









**PHOTOMETRIC  
CHEMICAL ANALYSIS**

**Vol. I**

**COLORIMETRY**



# PHOTOMETRIC CHEMICAL ANALYSIS

(COLORIMETRY AND NEPHELOMETRY)

BY

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VOLUME I

COLORIMETRY

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TO  
MY WIFE

“When you can measure what you are speaking about and express it in numbers, you know something about it, and when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind. It may be the beginning of knowledge, but you have scarcely in your thought advanced to the stage of a science.”

—LORD KELVIN.

## FOREWORD

IN the manufacture of almost any substance, material, or article, in industrial, pharmaceutical, medical, biological, and research chemistry, in times of peace as well as in times of war, analytical chemical methods, because they give information which is unobtainable by other means, have become of very great importance.

In all applied chemistry, the demand for accuracy in analytical methods varies with the material, application, or conditions in each case. Accuracy greater than the conditions or materials warrant, especially if it is at the expense of time, labor, or equipment, is never in demand, since it serves no useful purpose. The great demand in all fields of applied chemistry is for analytical methods of sufficient accuracy which require but little time for completion. In recent years another requisite has been added to that of sufficient accuracy and rapidity, namely that of sensitivity, so that extremely small amounts of substance can be analyzed or determined, if necessary.

Ordinary gravimetric analyses could and can comply with only one of the above qualifications, namely, accuracy. For rapidity and sensitivity they were not so designed and are by their nature not adaptable.

While here and there a volumetric method will fulfill all of the above requirements, especially that of rapidity and occasionally that of sensitivity at the cost of tediousness, the greatest progress in fulfilling these requirements has been made by means of photometric analysis—colorimetry and nephelometry. Photometric analytical methods have established new standards and new fields of application and research, owing to their high sensitivity, although in a few cases there is still room for improvement in accuracy and rapidity.

If the contributions of photometrical chemical analyses to industry and research consisted alone in their offering us the means of determining substances in very small amounts or in very high dilutions, this alone would justify their use and study. But in addition to this, their use in routine and control work as a primary means of analysis,



in industrial, pharmaceutical, biological, and medical work has been so great—references in the literature amounting to several thousand articles—and is growing so rapidly, that it is safe to say that photometric analyses are indispensably connected with the many fields of applied chemistry.

As photometric analysis is yet in its infancy, we are learning daily how to increase its efficacy, through improvement in accuracy, rapidity, and sensitivity. The study of the most sensitive reactions, the best media for their production and stabilization, involving in many cases the use of protective colloids, and finally the best methods and equipment for obtaining a photometric balance and determination, are all involved in photometric analytical research.

Many analytical chemists, not having seen or heard of photometric analyses in detail during their analytical training period, have been so accustomed to make their analytical procedures adaptable to either filtering, washing, drying, and weighing of precipitates, or to some volumetric titration, that photometric analytical procedures are new to them, their advantages unknown, or their accuracy underestimated.

While gravimetric and volumetric analyses will always be necessary for purposes of precise scientific measurements and perhaps also as standards for comparing shorter methods, yet since the application of chemistry involves so many of these photometric methods, no well-equipped student or chemist can afford to ignore the bases and principles of photometric analysis, its advantages, its accuracy, details of correct manipulation, the possible sources of errors, and the improvements that are, and can be, made in this very useful field of analytical chemistry.

For these reasons I believe that Professor Yoe's treatise on photometric analysis will be welcomed by students and workers as a great aid in the field of applied chemistry.

PHILIP ADOLPH KOBER

## PREFACE

THE rapid growth of colorimetry and nephelometry has created a demand for a comprehensive reference work on these two methods of chemical analysis. The use of color as a means of making quantitative chemical measurements is almost as old as the science of chemistry itself. The Greeks and Romans detected the presence of alkalis in natural waters by the decoloration of red wine. Keates is said to have been the first person to determine copper colorimetrically. This was done almost a century ago. During the past twenty-five years many new colorimetric methods have been developed, so that now most of the more common elements, a number of the less common ones, and many organic compounds may be determined by means of the colorimeter. The literature numbers several thousand references.

Nephelometry, on the other hand, is a comparatively new science. It had its beginning in the nineties when Richards used it as a means of making corrections in certain atomic-weight determinations. But it was not until 1912 that nephelometry as an analytical method was developed by Kober, and almost at the same time (1913), but entirely independently, by Bloor. Since that time nephelometry has made rapid strides both in the development of new methods and in the improvement of apparatus. Kleinmann in Germany has made important contributions within the past few years. Although nephelometry as an analytical method is in its infancy, it is rapidly growing, and already a literature of several hundred papers exists.

In view of the widespread application of colorimetry and the extensive development of nephelometry, it seems to the author that the time is now ripe for the theoretical consideration and correlation that these two sister methods of chemical analysis deserve. It is hoped that the chapters devoted to the actual operation of the instruments, the various methods of computing results, and the description in detail of representative analyses will clearly demonstrate to the chemist and biological worker the speed and accuracy obtainable by the use of photometric methods.

Furthermore, much progress in the sciences and the arts depends upon accurate determinations, and often upon rapid determinations, of amounts of substances that are too small to be estimated by gravimetric or volumetric analysis. From the point of view of rapidity, and for use in micro-analysis, colorimetry and nephelometry possess decided advantages; in fact, with the exception of the interferometer, the use of these methods is the only advance thus far made in this direction. As a matter of convenience, colorimetry has been treated in Volume I and nephelometry in Volume II. The second volume will appear within the next few months.

As this is the first extensive treatment of the subject, the author aims to cover a number of topics not ordinarily treated. This book has been written for both the advanced student in chemistry and the research worker. An extensive bibliography, arranged alphabetically by subject and chronologically under each subject, has been included. Many of the references contain a brief abstract, while others have been recorded with only the subject heading. It must not be assumed, however, that the relative importance of an article is indicated by the length of its abstract. Indeed, many excellent papers have been recorded with only their titles or subject headings.

It has been the aim of the author to make the bibliography an accurate and fairly complete survey of the literature on photometric chemical analysis. No doubt there are a number of articles that have escaped his attention, and he hopes that the users of the bibliography will call his attention to omissions in order that these may be included in a future edition. Also, notice of any errors will be greatly appreciated.

The author earnestly hopes that workers in this field will cooperate in improving (and "weeding out" if necessary) some of the less accurate and imperfectly studied methods given in this treatise. It is mainly with this idea in mind that these procedures have been included. As far as possible, the author has endeavored to state the limits of accuracy of each method, but, unfortunately, such data are not available at present for a number of the procedures; in others the data are given only for very limited applications. All chemical factors are based upon the International Atomic Weights for 1925.

The author owes a special debt of gratitude to Dr. Philip A. Kober, who read the entire manuscript for both volumes and made many helpful suggestions. In addition to this, Dr. Kober translated a

number of Dr. Kleinmann's papers, checked about a hundred references in the bibliography, and contributed a chapter on Directions for Using a Precision Colorimeter.

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J. H. Y.

UNIVERSITY, VIRGINIA,  
January, 1928.

# CONTENTS

## VOLUME I

### COLORIMETRY

#### PART I

#### GENERAL PRINCIPLES, APPARATUS, CALCULATIONS, CALIBRATION AND CORRECTION CURVES, ERRORS, COLLOIDS, AND DIRECTIONS FOR USING A PRECISION COLORIMETER

##### CHAPTER I

	PAGE
GENERAL PRINCIPLES.....	1-9
Standard-series Method, 3. Dilution Method, 4. Duplication Method, 4.	
Balancing Method, 4. Requirements of the Colorimetric Method, 5.	
Accuracy of Colorimetric Methods, 7. Speed of Colorimetric Methods, 7.	
Limits of Application of Colorimetric Methods, 8.	

##### CHAPTER II

COLORIMETRIC APPARATUS.....	10 51
Bottles, 12. Nessler Tubes, 12. Eggertz Tubes, 13. Julian Tubes, 14.	
Hehner Cylinders, 14. Color Camera, 15. Steiger Colorimeter, 15.	
Schreiner Colorimeter, 18. Kennicott-Campbell-Hurley Colorimeter, 21.	
Nessler Tube Colorimeter, 24. Bock-Benedict Colorimeter, 26. Kober	
Colorimeter, 28. Kober Precision Colorimeter-nephelometer, 32. Duboscq	
Colorimeter—Bausch & Lomb Model, 32. Duboscq Small or Biological	
Colorimeter—Bausch & Lomb Models, 38. Duboscq Colorimeter—E.	
Leitz Model, 40. Duboscq Colorimeter—Spencer Model, 42. Schmidt &	
Haensch Plunger Colorimeter No. 3 <i>a</i> —Duboscq System, 43. Kleinmann	
Microcolorimeter, 45. White Colorimeter, 48. Improved Bausch &	
Lomb Colorimeter Lamp, 50. New Palo Daylight Lamp—Cyllen Model,	
51. Cooper Hewitt Mercury Light, 51. Schmidt & Haensch Sphere	
Illuminating Device, 51. Klett Result Table, 51.	

##### CHAPTER III

CALCULATION OF RESULTS.....	52-69
Standard-series Method, 52. Dilution Method, 52. Duplication Method,	
53. Balancing Method, 54. Simplified Calculations in Colorimetry, 55.	
Tables of General Application for Verifying Complete Colorimetric Cal-	
culations, 57.	

## CHAPTER IV

	PAGE
COLORIMETER CALIBRATION AND CORRECTION CURVES.....	70-82

## CHAPTER V

ERRORS IN COLORIMETRY.....	83-89
Variable Sensitiveness of Vision to Different Concentrations, 83. Imperfect Susceptibility to Color, 83. Fatigue and Eye Strain, 84. "Fallacies in Colorimetry." Discussion by Dehn, 84. Different Optical Distributions, 84. Disturbances of Equilibria Resulting from Ionization, 87. Disturbances of Equilibria Resulting from Hydrolysis, 87. Disturbances Resulting from Chromoisomerization, 89. General Conclusions, 89.	

## CHAPTER VI

COLLOIDS AND COLORIMETRIC STABILIZERS.....	91-102
Protective Colloids and Other "Stabilizers," 91. Effect of Emulsoids on Colored Solutions, 93. The Solution Theory of Colloid Stability, 95. Interference Eliminators, 96. Nesslerization, 96. Peptization, 97. Color and Degree of Dispersion, 97. Concentration by Adsorption on a Crystalline Solid, 99. Concentration by Co-precipitation with a "Collector," 100. Factors that May Influence the Size of Colloid Particles, 102.	

## CHAPTER VII

GENERAL DIRECTIONS FOR USING A PRECISION COLORIMETER.....	103-108
The Choice of Instrument, 103. Specifications of a Plunger Type of Instrument, 103. Care of a Precision Instrument, 105. Source of Light, 106. Precautions to be Taken Relative to the Observer, 106. Method of Obtaining a Photometric Balance, 107.	

## PART II

## INORGANIC

## CHAPTER VIII

ALUMINUM.....	109-122
Determination of Aluminum by "Aluminon" (the Ammonium Salt of Aurin Tricarboxylic Acid), 109. Determination of Aluminum in Non-ferrous Material by "Aluminon," 114. Determination of Aluminum by Alizarin S, 119. Determination of Aluminum by Hematoxylin, 121.	

## CHAPTER IX

ANTIMONY AND ARSENIC.....	123-130
Determination of Antimony as the Sulfide, 123. Determination of Arsenic by Formation of a Stain on Mercuric Bromide Paper by Arsine, 125. Determination of Arsenic by Silver Nitrate, 128. Determination of Arsenic by Quinine Arsenomolybdate, 128.	

# CONTENTS

xv

## CHAPTER X

	PAGE
BISMUTH, BORON, AND BROMINE.....	131-138
Determination of Bismuth as the Iodide, 131. Determination of Boric Acid by Turmeric Paper, 133. Determination of Boric Acid by Curcumin, 135. Determination of Bromine, 137.	

## CHAPTER XI

CALCIUM.....	139-147
Determination of Calcium by Alizarin, 139. Determination of Calcium as the Oleate, 143. Determination of Calcium in Urine, 143. Determination of Calcium in Feces, 146.	

## CHAPTER XII

CARBON AND CYANIDE.....	148-156
Determination of Carbon in Steel, 148. Determination of Hydrogen Cyanide by Formation of Prussian Blue, 151. Determination of Cyanide by Conversion into Thiocyanate and Formation of Ferric Thiocyanate, 154.	

## CHAPTER XIII

CHLORINE, CHLORATE, AND PERCHLORATE.....	157-164
Determination of Free Chlorine by Ortho-tolidine, 157. Determination of Chlorides in Blood, 159. Determination of Chlorates by Aniline Chloride, 162. Determination of Perchlorates by Nitrosodimethylaniline, 163.	

## CHAPTER XIV

CHROMIUM.....	165-170
Determination of Chromium by Disodium 1, 8-Dihydroxynaphthalene-3, 6-Disulfonate, 165. Determination of Chromium by the Diphenylsemicarbazide Reaction, 166. Determination of Chromium as Chromate, 167. Determination of Chromium as Dichromate, 169.	

## CHAPTER XV

COBALT.....	171-175
Determination of Cobalt by Extraction with Amyl Alcohol, 171. Determination of Cobalt as the Chloride in Concentrated Hydrochloric Acid, 172. Determination of Cobalt by $\alpha$ Nitroso- $\beta$ -Naphthol, 173.	

## CHAPTER XVI

COPPER.....	176-185
Determination of Copper by Ammonia, 176. Determination of Copper as the Sulfide, 178. Determination of Copper as the Chloride in Concentrated Hydrochloric Acid, 179. Determination of Copper as the Bromide, 181. Determination of Copper by Potassium Ferrocyanide, 182. Determination of Copper by Salicylic Acid, 183. Determination of Copper by Ethyl Xanthate, 184.	

## CHAPTER XVII

FLUORINE.....	186-190
Determination of Fluorine by its Bleaching Action on an Oxidized Titanium Solution, 186.	



## CHAPTER XVIII

	PAGE
GOLD.....	191-197
Determination of Gold by Decomposition of the Cyanide by Potassium Bromate and Sulfuric Acid, 191. Determination of Gold by Decomposition of the Cyanide by Sodium Bromide and Sodium Peroxide, 191. Determination of Gold by Decomposition of the Cyanide by Ammonia, 192. Determination of Gold by Metaphenylenediamine, 192. Doring's Method for the Determination of Gold, 193. Dowsett's Method for the Determination of Gold, 194. Prister's Method for the Determination of Gold, 195. Rose's Method for the Determination of Gold, 196.	

## CHAPTER XIX

HYDROGEN ION.....	198-217
The Hydrogen-ion Exponent Scale, or pH Scale, 199. Buffer Action, 201. Unbuffered Solutions, 204. Making Hydrogen-ion Determinations, 205. Procedure for Using the LaMotte Block Comparator, 210. LaMotte Hydrogen-ion Testing Set, 213. Some Applications of Hydrogen-ion Control, 216.	

## CHAPTER XX

IRON.....	218-249
Determination of Iron as the Sulfocyanate (Thiocyanate), Method of Stokes and Cain, 218. Determination of Iron as the Sulfocyanate, Method of Mellor, 237. Determination of Iron as Ferric Chloride in Concentrated Hydrochloric Acid, 238. Determination of Iron as Sulfide, 239. Determination of Iron by Potassium Ferrocyanide, 241. Determination of Ferrous Iron by Potassium Ferricyanide, 242. Determination of Iron by Salicylic Acid, 243. Determination of Iron by Acetylacetone, 245. Determination of Iron by Dimethylglyoxime, 248.	

## CHAPTER XXI

LEAD.....	250-263
Determination of Lead as the Sulfide, 250. Determination of Lead as the Chromate, 254. Determination of Lead by Diphenyl Carbazide, 255. Determination of Lead by Hematin, 257. Determination of Lead by Aniline, 257. Determination of Lead by Sodium Bisulfite, 257. Determination of Lead in Urine and Feces, 258.	

## CHAPTER XXII

MAGNESIUM.....	264-270
Determination of Magnesium by the Bell-Doisy Reaction, 264. Determination of Magnesium by Separation as Magnesium Ammonium Phosphate and Estimation of the Phosphate as Phosphomolybdate, 266. Determination of Magnesium as the Oleate, 270.	

## CHAPTER XXIII

MANGANESE.....	271-281
Determination of Manganese by Oxidation with Persulfate, 271. Determination of Manganese by Oxidation with Periodate, 273. Determination of Manganese in Water by Sodium Bismuthate, 279.	

## CHAPTER XXIV

	PAGE
MERCURY AND MOLYBDENUM.....	282-294
Determination of Mercury as Sulfide, 282. Determination of Molybdenum by Hydrogen Peroxide, 283. Determination of Molybdenum by Tannic Acid, 284. Determination of Molybdenum in Tungsten, 285. Determination of Molybdenum in Steel, 292.	

## CHAPTER XXV

NICKEL.....	295-305
Determination of Nickel as the Dimethylglyoxime, 295. Determination of Nickel by Potassium Thiocarbonate, 296. Determination of Nickel by Potassium Di-thio-oxalate, 298. Determination of Nickel as the Chloride in Concentrated Hydrochloric Acid, 303.	

## CHAPTER XXVI

NITROGEN (AMMONIA, NITRITE, AND NITRATE).....	306-322
Determination of Ammonia by Nessler's Reagent, 306. Determination of Ammonia by Phenol and Sodium Hypochlorite, 307. Determination of Nitrite by Sulfanilic Acid and $\alpha$ -Naphthylamine, 308. Determination of Nitrite by $\alpha$ -Naphthylamine Hydrochloride, 309. Determination of Nitrite by Metaphenylenediamine, 310. Determination of Nitrite by Dimethylaniline, 311. Determination of Nitrite by Antipyrin, 311. Determination of Nitrite by Zinc Iodide Starch Solution, 312. Determination of Nitrate by Phenoldisulfonic Acid, 313. Determination of Nitrate by Diphenylbenzidine, 316. Determination of Nitrate by Reduction and Nesslerization, 317. Determination of Nitrate by Brucine, 318. Determination of Nitrate by Pyrogallol, 319. Determination of Nitrate by Strychnine Sulfate, 320.	

## CHAPTER XXVII

OXYGEN (FREE OXYGEN, HYDROGEN PEROXIDE, AND WATER).....	323-335
Determination of Dissolved Oxygen in Water by Cuprous Ammonium Chloride, Method of Ramsay and Hombray, 323. Determination of Dissolved Oxygen in Water by Cuprous Ammonium Chloride, Method of Frankforter <i>et al.</i> , 326. Determination of Oxygen by "Adurol," 330. Determination of Hydrogen Peroxide by its Oxidizing Action on Ferrous Iron, 332. Determination of Hydrogen Peroxide by Ammonium Molybdate, 333. Determination of Water in "Absolute" Ethyl Alcohol, 334.	

## CHAPTER XXVIII

PHOSPHORUS.....	336-354
Determination of Phosphorus as Phosphomolybdate, 336. Determination of Phosphorus by Separation as Magnesium Ammonium Phosphate and Estimation of the Phosphate as Phosphomolybdate, 337. Determination of Phosphorus by Precipitation as Phosphomolybdate and Reduction with Hydrazine Sulfate, 341. Determination of Phosphorus as Phosphovanadomolybdate, 342. Determination of Phosphorus by Molybdic Acid-quinine Reagent, 343. Determination of Phosphorus by Hydrogen Sulfide, 344. Determination of Phosphorus in Urine and Blood by the	

	PAGE
Bell-Doisy-Briggs Method, 346. Determination of Phosphorus in Blood and Urine by the Fiske and Subbarow Method, 348. Determination of Inorganic Phosphate in Blood by the Benedict and Theis Method, 353. Determination of Phosphate and Silica in the Presence of Each Other by Ammonium Molybdate, 354.	
CHAPTER XXIX	
PLATINUM.....	355-356
Determination of Platinum by Potassium Iodide, 355.	
CHAPTER XXX	
POTASSIUM.....	357-361
Determination of Potassium by Precipitation with Chlorplatinic Acid and Reduction with Stannous Chloride, 357. Determination of Potassium by the Addition of Potassium Iodide to the Chlorplatinic, 359.	
CHAPTER XXXI	
SELENIUM AND SILICON.....	365-371
Determination of Selenium as Selenious Acid by Potassium Iodide, 365. Determination of Silicon by Reduction of the Silicomolybdate by Sodium Sulfite, 366. Determination of Silica and Phosphate in the Presence of Each Other by Ammonium molybdate, 369.	
CHAPTER XXXII	
SULFUR AND HYDROGEN SULFIDE.....	372-376
Determination of Sulfur as Lead Sulfide, 372. Determination of Sulfur by the Methylene Blue Method, 373. Determination of Sulfur by the Action of Hydrogen Sulfide on Arsenious Oxide Paper, 375. Determination of Hydrogen Sulfide by Formation of Methylene Blue, 375.	
CHAPTER XXXIII	
TITANIUM.....	377-386
Determination of Titanium in Iron and Steel, 377. Determination of Titanium by Thymol, 381. Determination of Titanium by Hydrogen Peroxide (Sodium Peroxide Fusion), 384. Methyl Orange as a Permanent Standard for the Determination of Titanium by the Peroxide Method, 386.	
CHAPTER XXXIV	
TUNGSTEN AND VANADIUM.....	387-395
Determination of Tungsten as the Oxide in Colloidal Suspension, 387. Determination of Vanadium in Iron and Steel, 388. McCabe's Method for the Determination of Vanadium in Iron and Steel Containing Tungsten, Chromium, Titanium, and Molybdenum, 391. Determination of Vanadium by Strychnine, 393.	
CHAPTER XXXV	
ZINC.....	396-399
Determination of Zinc by Resorcinol, 396. Determination of Zinc by the Potassium Ferrocyanide Method (Turbidimetric Method), 397.	

# CONTENTS

xix

## PART III

### ORGANIC

#### CHAPTER XXXVI

	PAGE
ACETALDEHYDE, ACETYLENE, AND ACROLEIN.....	401-407
Determination of Acetaldehyde by Fuchsine-sulfurous Acid, 401. Determination of Acetylene by Ammoniacal Cuprous Chloride, 402. Determination of Acrolein by Fuchsine-sulfurous Acid, 407.	

#### CHAPTER XXXVII

ADRENALINE.....	408-409
Determination of Adrenaline, 408.	

#### CHAPTER XXXVIII

ANILINE.....	410-411
Determination of Aniline Vapor, 410.	

#### CHAPTER XXXIX

BENZALDEHYDE AND CITRAL.....	412-414
Determination of Benzaldehyde by Fuchsine-sulfurous Acid, 412. Determination of Citral by Fuchsine-sulfurous Acid, 414.	

#### CHAPTER XL

DYES.....	415-417
Determination of Certain Vat Dyes by Reduction with Alkaline Sodium Hyposulfite, 415.	

#### CHAPTER XLI

FORMALDEHYDE AND FURFURAL.....	418-422
Determination of Formaldehyde by Morphine Sulfate, 418. Determination of Furfural by Orcinol, 419. Determination of Furfural by Aniline, 421.	

#### CHAPTER XLII

ORGANIC SUBSTANCES (MISCELLANEOUS) AND PENTOSE.....	423-428
Determination of Organic Substances by Potassium Bichromate-sulfuric Acid, 423. Determination of Pentose in Free or Combined Form, 426.	

#### CHAPTER XLIII

PHENOL AND SALICYLIC ACID.....	429-434
Determination of Phenols by the Folin and Denis Reagent, 429. Determination of Phenol by Millon's Reagent, 431. Determination of Salicylic Acid by Iron Alum, 432. Determination of Salicylic Acid by Fehling's Solution, 433.	

## CHAPTER XLIV

TANNIN AND THIOPHEN.....	PAGE 435-436
Determination of Tannin, 435. Determination of Thiophen in Crude Benzene, 436.	

## CHAPTER XLV

VANILLIN.....	437-441
Determination of Vanillin, 437.	

*PART IV*

## BIOLOGICAL

## CHAPTER XLVI

BLOOD ANALYSIS.....	443-468
Method of Drawing Blood for Analysis, 443. Preparation of Protein-free Blood Filtrate, 444. Determination of Non-protein Nitrogen, 446. Determination of Urea, 451. Determination of Pre-formed Creatinine, 457. Determination of Creatine, 461. Determination of Uric Acid by Direct Colorimetric Methods, 462. Determination of Amino-acid Nitrogen, 467.	

## CHAPTER XLVII

BLOOD ANALYSIS (Continued).....	469-499
Determination of Sugar by Folin's Modification of the Folin-Wu Method, 469. Determination of Sugar by Benedict's Modification of the Folin-Wu Method, 471. Determination of Sugar by Benedict's Picric Acid Method, 472. Determination of Cholesterol and Fatty Acids by the Method of Bloor, Pelkan, and Allen, 474. Determination of Cholesterol by the Method of Myers and Wardell Modified, 476. Determination of Lipoid Phosphorus (Lecithin) by the Methods of Bloor, and Benedict and Theis, 478. Determination of Bile Pigment in Serum. Icteric Index, 479. The Van den Bergh Test for Bile Pigment in Serum, 480. Determination of Bile Salts, 480. Determination of Phenols, 481. Determination of Hemoglobin by the Newcomer Method, 482. Determination of Hemoglobin by the Acid Hematin Method of Cohen and Smith, 487. Determination of Iron in Biological Material, 491. Determination of Hydrogen Ion, 492.	

## CHAPTER XLVIII

URINE ANALYSIS.....	500-517
Determination of Total Nitrogen in Urine, 500. Determination of Urea, 510. Determination of Ammonia, 513. Determination of Amino-acid Nitrogen by the Method of Folin, 515.	

## CHAPTER XLIX

URINE ANALYSIS (Continued).....	518-534
Determination of Creatinine, 518. Determination of Creatine, 519. Determination of Uric Acid by the Benedict and Franke Direct Colorimetric Method, 522. Determination of Uric Acid by the Folin and Wu Colori-	

# CONTENTS

xxi

metric Method, 522. Determination of Sugar by the Method of Sumner, 524. Determination of Sugar by the Method of Benedict and Osterberg, 525. Determination of Proteins by the Method of Folin and Denis, 527. Determination of Phenols by the Method of Folin and Denis, 528. Determination of Manganese by the Method of McCrackan and Passamaneck, 531. Determination of Hydrogen Ion, 533.

PAGE

## PART V

## BIBLIOGRAPHY

## PART VI

## TABLES

LOGARITHMS.....	732-736
AUTHOR INDEX.....	737
SUBJECT INDEX.....	759



These errata are inserted in Volume II for the benefit of those who own a copy of Volume I.

## ERRATA

### VOLUME I

#### PAGE

- 5, footnote, "J. Lab. Clin. Med." should appear only once.  
 6, line 20, omit "reasonably."  
 28, line 14, "scales" should read "scale."  
 37, line 20, "plunger" should read "plungers."  
 37, line 22, "on both sides" should read "between the two halves."  
 80, the first equation should read  $S = \frac{Y}{X} - \frac{(1-X)YK}{X^2}$ .  
 81, the first equation should have "=" after S instead of "-."  
 87, in footnote 12, last line, "showed" should read "shown."  
 95, line 37, " $xSb_2S_3 \cdot yS^-$ " should read " $xSb_2S_3yS^-$ ."  
 97, line 3 from bottom, "trisulfide" should read "sulfide."  
 134, line 18, and page 136, line 3, formula for borax should be  
     " $Na_2B_4O_7 \cdot 10H_2O$ ."  
 167, line 9, "9.2" should be "0.2."  
 248, the formula for dimethylglyoxime should be  $CH_3 - \overset{\overset{||}{NOH}}{C} - \overset{\overset{||}{NOH}}{C} - CH_3$   
 378, line 10, "other" should read "ether."  
 380, Note 1, line 5, "residue" should follow "insoluble."  
 394, line 6, "acid" should follow "nitric."  
 445, line 2, "poassium" should read "potassium."





# COLORIMETRY

## PART I

### GENERAL PRINCIPLES, APPARATUS, CALCULATIONS, CALIBRATION AND CORRECTION CURVES, ERRORS, COLLOIDS, AND DIRECTIONS FOR USING A PRECISION COLORIMETER

## CHAPTER I

### GENERAL PRINCIPLES

It has long been the custom of chemists to classify all methods of analysis according to the technique employed, i.e., gravimetric analysis or volumetric analysis. If we classify the methods according to the physico-chemical principles involved, it will be found that practically all quantitative procedures may be grouped under the following important principles:<sup>1</sup>

1. Neutralization.
2. Solubility-product or saturation-value of the ion-concentration product.
3. Oxidation-reduction.
4. Colorimetry and nephelometry (photometric chemical analysis).
5. Evolution and measurement of gases.

In the present treatise we are concerned with the fourth principle listed above. *Photometric chemical analysis* may be defined as *analysis which depends upon a change in the amount or character of light due to a chemical reaction*. The change in the amount of light as understood in this treatise is that due to either absorption or reflection. Analysis made on the basis of absorption is usually called *colorimetry*, and in a

<sup>1</sup> Cf. H. A. Fales, *Inorganic Quantitative Analysis*, p. 5. The Century Co., New York, 1925.

few cases turbidimetry, while analysis based on reflected light is called *nephelometry*. A few colorimetric methods based upon the formation of color stains have also been included. As a matter of convenience, we shall treat colorimetry and nephelometry separately, the latter being discussed in Volume II.

The use of color as a means of determining the amount of a given substance present has long been employed, for example, in the determination of ammonium, nitrite, and nitrate nitrogen in water or of carbon in steel. Also, color may be referred to an absolute index of color value, for example, by use of the Lovibond Tintometer; or it may be determined by absolute analysis in terms of wave-length of dominant hue or its complement and the percentage of white, for example, monochromatic analysis by means of the Nutting Colorimeter.

Colorimetric methods have rapidly increased in number during the past twenty-five years, so that the list now includes many metals (aluminum, chromium, cobalt, copper, gold, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, titanium, tungsten, vanadium, zinc, etc.), many non-metals (arsenic as arsenate; boron as borate; carbon; hydrogen-ion; nitrogen as nitrite, nitrate, and ammonium; oxygen both free and as hydrogen peroxide; silicon as silica; sulfur as sulfide; phosphorus as phosphate; etc.), and a large number of organic substances which include nearly all classes of organic compounds (alcohols, aldehydes, organic acids, esters, phenols, carbohydrates, alkaloids, hemoglobin, etc.).

In general, colorimetric analysis consists in adding a reagent to a solution of the test substance in such a way as to produce a color. The basis of colorimetry may be stated as follows: When equal heights or thicknesses of two solutions give the same intensity of color, the concentrations of the solutions are said to be equal. When equal color intensity is obtained from different heights of two solutions, the assumption is often made, in accordance with Beer's law, that the concentrations are inversely proportional to the heights. While many colors follow Beer's law sufficiently closely, recent colorimetric work makes no assumption at all; but, as shown in Chapter IV, the amount of color for each concentration is obtained by standardization with each color under the conditions found in any particular determination.

As a matter of convenience, and also to insure greater precision and speed, special forms of apparatus have been developed for use in

colorimetry. Many of the more commonly used instruments are described in detail in Chapter II. The methods employed in matching colors may be grouped under four heads:

1. Standard series method.
2. Dilution method.
3. Duplication method.
4. Balancing method.

**Standard Series Method.**—In this method the sample solution contained in a glass tube (or cell) is diluted to a definite volume, mixed, and its color compared with a series of standards similarly prepared.

In some cases it is possible, and may be more convenient, to prepare a series of permanent standards by means of solutions and mixtures of solutions of certain colored inorganic salts, such as, for example, cobalt chloride, ferric chloride and copper sulfate.<sup>2</sup> If such a series is employed, each solution must, of course, be standardized against a known amount of the original substance and under *identical conditions* as maintained in the analysis of a sample of the substance. Care must be taken that the *tint* or *shade* of color is the same in the permanent standard as in a solution of the substance to be determined.

Sometimes it is a great convenience to use a series of colored glasses as standards. For example, cobalt glass has been found to match the blue color of certain vat dyes reduced by alkaline sodium hyposulfite.<sup>3</sup> The use of these permanent standards proved a great help in reaction velocity studies on these dyes where it was necessary to make rapid determinations every few minutes and, also, on account of the fact that the reduced dyes are readily oxidized by air, and therefore a freshly prepared standard would be required for each determination. The plates of glass were standardized against known weights of dye reduced under standard conditions and were always placed in the colorimeter in a *definite position* to guard against introducing an error due to any irregularity in the glass. In using colored glasses as permanent standards, great care must be taken that the *tint* of color exactly matches that of the test solution. For a more

<sup>2</sup> H. V. Army and C. H. Ring, *J. Franklin Inst.*, **180**, 199 (1915); *J. Ind. Eng. Chem.*, **8**, 309 (1916).

<sup>3</sup> J. H. Yoe and G. Edgar, *J. Phys. Chem.*, **27**, 65 (1923); J. H. Yoe, *ibid.*, **28**, 1211 (1924).

detailed discussion of permanent standards consult the references on standards given in the **Bibliography (Part V)**.

With a series of standards, the amount of substance in the sample is obtained directly, since it is equivalent to the amount contained (or represented) in the standard which it matches in color intensity.

**Dilution Method.**—If the sample and standard solutions, when placed in glass tubes (or cells) of the same diameter and observed *horizontally* through the tubes, have the same intensity of color, obviously their concentrations are identical. Usually the solutions do not match in color intensity, and the darker one is then diluted until a match is obtained when the two are viewed *horizontally* through the tubes, i.e., through the same thickness of liquid. This process of comparison is called the dilution method. When sample and standard solutions match, their concentrations are the same and, hence, the weights of the substance in the two solutions are directly proportional to the respective *volumes*.

**Duplication Method.**—This method is carried out as follows: The sample is placed in a glass tube (or cell), diluted to a definite volume, and mixed. Water is put in a similar vessel and the same reagent (or reagents) added as used to produce the color with the sample. The volume in this “blank” should be a little smaller than that of the sample solution. Next, a relatively concentrated standard solution of the substance being determined is run into the “blank” from a burette until its color matches that of the sample solution, the final observation being made after the “duplicate” has been brought up to the same volume as the sample, by the addition of distilled water, and thoroughly mixed. The amount of standard solution required to make the “duplicate” is a measure of the amount of substance in the sample solution.

**Balancing Method.**—This method consists in placing the sample solution (or an aliquot portion) in a flat-bottom graduated tube and then running into another similar tube a standard color solution until the color intensities of the two are the same when viewed *vertically* through the length of the columns of liquids. When thus “balanced,” the concentrations of the two solutions are *inversely* proportional to their *heights* (not volumes) in the tubes. Schreiner<sup>4</sup> points out that “curiously enough, the graduation into cubic centimeters has been carried over to the cylinders used in many of these instruments

<sup>4</sup> J. Am. Chem. Soc., **27**, 1198 (1905).

(balancing or plunger type colorimeters) when it is perfectly obvious that it is the height of the standard liquid which determines the strength of the unknown solution." Of course, if both the sample and standard tubes have the same diameter and the bore is uniform throughout in each tube, then the two concentrations are *also* inversely proportional to their *volumes* when the colors are matched by the balancing method. Such uniformity in color tubes is hard to obtain, and is unnecessary when the balancing method is employed, unless the highest degree of precision is required. Moreover, the use of the cubic centimeter scale in graduating the balancing or plunger type colorimetric apparatus is wrong in principle and therefore should not be employed. A rational graduation into scale divisions (say, centimeters) independent of capacity or uniformity of bore of the tube should be used.

Instead of placing the sample solution in the tube and changing the height of the standard column until the color intensity is the same as that in the unknown, a measured height of standard solution may be placed in one tube and the sample solution run into the other until a color match is obtained.

The balancing method is by far the speediest of the four procedures above mentioned. It is also, in general, the most accurate, provided the proper conditions are observed.

The change in the height of a solution has been accomplished in a variety of ways, e.g., (1) by dropping from a burette into one of the comparison tubes, (2) by providing one or both of the tubes with a stopcock near the bottom, (3) by connecting, by means of a side tube at the bottom, with a reservoir which permits moving the solution up and down at will, and (4) by changing the height by means of an immersion prism or tube. These types of instruments are described in detail in the chapter on Colorimetric Apparatus (Chapter II).

**Requirements of the Colorimetric Method.**<sup>5</sup>—In order to make possible the employment of a colorimetric method, certain conditions must be fulfilled, chief among which are the following:

1. The color produced by the reagent must be characteristic of the test substance, or, in case certain other substances produce the same color as does the test substance, these must be known to be absent.

2. The color produced by the reagent and test substance must be

<sup>5</sup> J. H. Yoe, J. Lab. Clin. Med., J. Lab. Clin. Med. **13**, 139 (1927).

the only color present in the solution. In some cases the presence of a very small amount of a foreign colored substance in the sample solution may be compensated by using a standard with the *same concentration of the foreign substance*. For example, a titanium solution containing a small amount of ferric salts may be accurately matched against a standard titanium solution to which has been added ferric ions sufficient to equal the concentration of the latter in the test solution.

3. The sample solution must be colorless, or, if colored, this color must be removed by the reagent or by some other step in the procedure.

4. The sample solution must not contain any foreign substance which will give a color or precipitate with the reagent.

5. The color produced by the reagent must be reasonably permanent, i.e., it must not fade so rapidly that an accurate color comparison is impossible. Under certain well-defined conditions it is sometimes possible to employ a fairly unstable color and still obtain a satisfactory quantitative measurement. Such cases will be treated in the text in their respective procedures.

6. Neither the *intensity* nor the *tint* of the color produced by the reagent and test substance must be affected by the presence of reasonably relatively high concentrations of electrolytes likely to be present. In certain cases it is necessary to adjust very carefully the hydrogen-ion concentration before an accurate color comparison can be made.

To the above may be added certain other conditions which are desirable in a colorimetric method but which are not always required:

1. The intensity of the color should be directly proportional to the concentration of the test substance.

2. The color should be one easy to distinguish and to match; for example, blue, red, green, etc. In this connection it must be remembered that an operator may have a dull or imperfect susceptibility to one color and still be able to match other colors with great precision. It is therefore important that he test himself thoroughly for each color by matching a standard against itself in several degrees of intensity. If concordant results are not obtained with a certain color, it is useless for him to go further with this color.

3. The method should be rapid, accurate, and sensitive. Frequently one, or maybe two, of these qualities are sacrificed in order to attain the more desired third quality. In general, colorimetric methods are rapid and accurate and often are delicate enough to

determine quantitatively one part of the test substance in several hundred thousand parts of water. Some are so sensitive that one part of test substance may be detected in a hundred million parts of water.

**Accuracy of Colorimetric Methods.**—No general statement can be made as to the accuracy of colorimetric methods. Some colorimetric determinations have been brought to such a high degree of perfection that they far surpass gravimetric or volumetric determinations in accuracy. On the other hand, many colorimetric methods are only rough approximations. These approximate methods, however, serve a purpose, for in such cases we frequently have no other means of determining the substances; or, as often happens, a very *rapid* method may be necessary, and a colorimetric procedure, although its results are only approximate, may meet this requirement. It is between the above two extremes of accuracy that most of the colorimetric methods lie. Attention has often been called to the extreme degree of accuracy attainable in colorimetric methods when properly carried out. . . . "There is little doubt that their accuracy is more frequently underestimated than overestimated. This is due to a number of causes, chief among which are the inability on the part of many persons to judge colors accurately, contamination while making the tests, the use of impure reagents, and the employment of faulty apparatus. Practice will do a great deal to enable one to make good comparisons, but it can never make up for a dulled or imperfect susceptibility to color."<sup>6</sup> Great attention should be given to this point in using colorimetric methods. See page 83.

**Speed of Colorimetric Methods.**—As in the case of accuracy, colorimetric methods vary widely from the standpoint of speed. Some are extremely rapid, requiring only a few minutes, while others are very slow and tedious, especially if the highest degree of accuracy is desired. Often *accuracy* is sacrificed for *speed*. "In devising colorimetric methods there have been two main objects throughout, namely, speed and the ability to estimate small amounts, both of which are common to many of the methods, but not necessarily so. A colorimetric method may have speed and yet not be capable of estimating very small amounts. Speed is, through necessity perhaps, of the greatest importance to the works chemist and to the busy analyst. The ability to estimate very small amounts of material, however,

<sup>6</sup> O. Schreiner, J. Am. Chem. Soc., **27**, 1196 (1905).



is of the greatest importance to the modern investigator in the fields of pure and applied sciences, and to him speed is of only secondary importance if indeed he values it at all. Some of the more recently devised colorimetric methods are for this very reason fully as laborious and perhaps even more tedious than the gravimetric methods, and their one virtue lies in the fact that they can be used in determining amounts so small that gravimetric methods fail, and, hence, they present a means of attacking problems which hitherto had been impossible of investigation.

"The choice whether a gravimetric [or a volumetric] or a colorimetric method should be used in a given case lies therefore almost wholly within that class of colorimetric methods which have been devised for speed, and the question in this case is usually only one of comparative accuracy as balanced by the gain in time. This gain in time may, however, be of the utmost importance, not only in a works laboratory, but also in a scientific investigation where it is necessary to know the amount of a substance present at any given time in the course of an experiment."<sup>7</sup>

In spite of certain limitations "the colorimeter is coming into use more and more every day because of its answer to the demand in nearly every laboratory for speed. Colorimetric methods, used because of their speed, give results in five minutes to one hour from the time the test is begun which is in all cases less than half the time similar tests could be made by other methods [except perhaps potentiometric methods]. A leading brass manufactory of the country [United States] obtains an analysis of its brass from the laboratory within forty-five minutes after the delivery of the sample. Of the five constituents determined, four are determined by colorimetric methods."<sup>8</sup>

**Limits of Application of Colorimetric Methods.**—In general, a colorimetric method cannot be used when more than one or two per cent of the substance being determined is present without resorting to aliquot parts and using a portion of the solution of the sample instead of the whole. In the latter case it is, of course, necessary to measure the aliquot part as accurately as the sample was measured, otherwise the final result will be in error.

As for the lower limit of application, it may again be pointed out

<sup>7</sup> O. Schreiner, *J. Am. Chem. Soc.*, **27**, 1194 (1905).

<sup>8</sup> F. D. Snell, *Colorimetric Analysis*, p. 3. D. Van Nostrand Co., New York, 1921.

that many colorimetric reactions are sensitive enough to detect one part of test substance in several million parts of water and some will detect one part in a hundred million parts of water. Hence, by using a large weight of the sample material, or a large volume in the case of solutions and then concentrating by evaporation, extremely small amounts may be determined. Of course, the size of sample that can be handled reaches a practical limit. For example, a long time is required for concentrating large volumes of liquids and great care must be taken to prevent contamination from dust particles, vessels, etc. Furthermore, various salts may crystallize out during evaporation and occlude some of the substance being sought, or a certain constituent which has no effect in the dilute sample may interfere when its concentration is increased.

In spite of the many requirements imposed upon colorimetric methods, it may be said that, in general, they are applicable to concentrations of one or two per cent down to one part in a hundred million, but these limits may be extended under proper conditions as pointed out above.

## CHAPTER II

### COLORIMETRIC APPARATUS

COLORIMETRIC apparatus may be divided into two types according to the method of comparison:

1. Apparatus used in the (*a*) standard series, (*b*) dilution, and (*c*) duplication methods.
2. Apparatus used in the balancing method.

Apparatus of the first type is generally very simple, consisting of bottles, Nessler tubes, Eggertz tubes, Julian tubes, the color camera, etc. The balancing method requires more elaborately constructed apparatus but the use of it is the simplest. These instruments range from the simple Hahn cylinders to the elaborate plunger type and wedge type colorimeters, having special optical arrangements which enable high precision in matching colors.

The plunger type colorimeter with the two halves of the field of view illuminated by the light passing through the unknown and standard solutions, respectively, was first announced by Jules Duboscq, of Paris, in 1854, and improved modifications of this instrument have been made in recent years by various manufacturers, particularly in the United States.

Special forms of colorimeters, some of which are less elaborate and less expensive than the Duboscq type, have been developed. Among these may be mentioned the Schreiner Colorimeter for soil work; the Kennicott-Campbell-Hurley and the Nessler Tube Colorimeters for water analysis, rock analysis, steel analysis, analysis of alloys, etc.; the Saybolt Chromometer for oil testing; the Stammer Colorimeter used in sugar analysis for grading syrups, and estimating the decolorizing power of bone black and other clarifying agents, and for many other purposes for which the degree of color, and not determination of color-producing substance, is desired; and the Lovibond Tintometer for standardizing merchantable petroleum and other purposes.

The Duboscq, Bausch & Lomb-Duboscq, Leitz-Duboscq, Spencer-Duboscq, Kober, Bock-Benedict, Schmidt & Haensch, and Kleinmann colorimeters are designed particularly for biochemical and clinical work, such as the determination of creatinine, total nitrogen, and urea in urine, etc. and the determination of non-protein nitrogen, urea and ammonia in blood. These are instruments of high precision and are useful for work requiring the highest degree of accuracy, especially when only a small amount of sample is available.

Colorimeters of the wedge type are illustrated by White's Colorimeter, which is suitable for the analysis of ores and alloys containing fairly large amounts of the test substances, and by Myers' Bi-Colorimeter (three-wedge type) which has been constructed primarily for the determination of hydrogen-ion concentration.

For an absolute analysis of color the Nutting Colorimeter may be used. This instrument measures color in terms of wave-length of dominant hue or its complement and the percentage of white (monochromatic analysis). It is used in the analysis and specification of colors of liquids, powders, papers, textile fabrics, color screens, light source, etc.; for photometry (including spectrophotometry); and for determining visual sensibility, hue sensitivity, and purity sensibility. This instrument makes it possible to measure and record numerically a color so that at any future time, and with a different instrument if desired, it can be accurately reproduced from the record. The determination of color blindness and lesser abnormalities of color sensibility also come within its scope.

The Nutting Colorimeter is essentially a spectroscope with two additional arms, one to admit standard white light, the other, the light to be analyzed. For a description of this instrument and directions for using it, see U. S. Bureau of Standards, Bulletin 9, 1-5 (1913) and Physical Review [2], 4, 454-455. The original instrument has been modified considerably, the latest form being the Hilger New Model approved by Dr. Nutting.

It may be mentioned, finally, that aside from a few brief statements, analyses involving the use of a Tintometer (Lovibond, Wesson, etc.) or the Nutting Colorimeter (and similar instruments) will not be included in this treatise.

In the following pages of this chapter an attempt is made to describe accurately colorimetric apparatus, including a number of the commonly used colorimeters. For a description of apparatus for the

colorimetric determination of hydrogen-ion, see Chapter XIX. Colorimeters designed especially for the determination of hemoglobin are described in Chapter XLVII.

### COLORIMETRIC APPARATUS

**1. Bottles.**—For rapid work and in cases where the highest degree of accuracy is not necessary a series of bottles containing standard color solutions may be used to advantage. Such bottles should be carefully selected. They should be made of clear glass, uniform in thickness, and without flaws. All bottles should be uniform in size and shape. Bottles having flat and parallel sides are preferable.

When the series of standards is ready, the bottles may conveniently be arranged in a row, spaced at a distance equal to their diameter. The sample is treated in a similar bottle, diluted to the same volume as the standards, and then inserted in the gaps along the series of bottles until its color matches one of the standards. Frequently a sheet of white paper or a piece of white frosted glass held back of the bottles will aid the observer in matching the color. A uniform source of light is essential. Usually the light from the north sky (rich in blue) is best, or the light may be obtained from a lamp designed especially for colorimetric work. See page 50. A "blue sky" is 23.8 per cent red, 27.2 per cent green, and 49.0 per cent blue.

**2. Nessler Tubes.**—Figure 1. These tubes must be made of clear glass, must be uniform in bore, and have polished, flat bottoms.

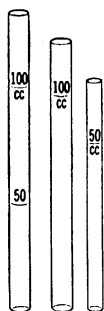


FIG. 1.  
Nessler Tubes

Two sizes are in common use: tubes 50 cc. and 100 cc. capacity. The dimensions and capacities established by the American Public Health Association are as follows: The 50 cc. mark on 50 cc. tubes should be about 210 mm. high and the 100 cc. mark on 100 cc. tubes should be about 325 mm. high. In a set of tubes the marks must be the same height or not differ more than 6 mm. 100 cc. tubes may also be marked at 50 cc.

The A. P. H. A. tube is the tall form Nessler tube. In the low form Nessler tube the 50 cc. mark of the 50 cc. tube is about 120 mm. high and the 100 cc. mark of the 100 cc. tube is about 150 mm. high.

In color matching the Nessler tubes may be placed in a wooden

box (Fig. 2) so arranged that the light is reflected from the bottom up through the tubes, the latter resting on a rack having a false

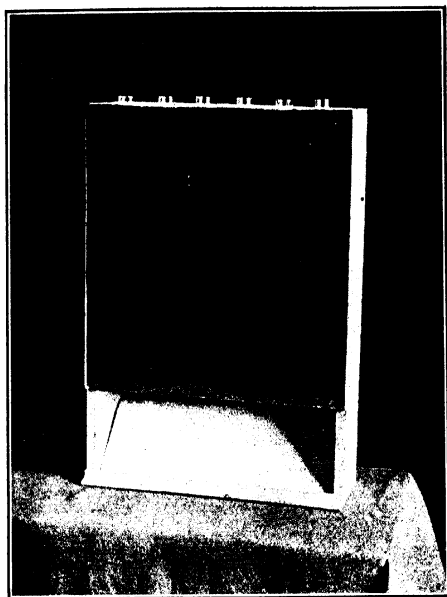


FIG. 2.—Nessler Tube Box

bottom. The box should be painted inside a dull black. Or, the tubes may be arranged in a specially constructed rack (Fig. 3). This rack is made of wood finished in dull black and has an opal glass reflector at the bottom set at an angle. The tubes rest on a metallic ledge under the lower deck so that their bottoms do not come in contact with the opal glass reflector. This arrangement prevents introducing errors due to shadows.

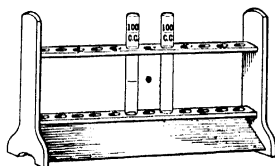


FIG. 3.—Nessler Tube Rack

**3. Eggertz Tubes.**—Figure 4. Eggertz tubes are especially adapted to the colorimetric method for the determination of carbon

and manganese in steel. They are long tubes of small bore and are graduated to tenths of a cubic centimeter. The graduation marks and figures should be small and must be as accurately made as those of a burette. The cubic centimeter marks may be 4 mm. long, but the subdivisions and figures should not be over 2 mm. long. In a set of two or three tubes, all graduations should coincide with each other, thus proving the inside diameter is uniform throughout the set. Only carefully selected tubing made of colorless glass should be used. The tubing should be free of scratches, fine black lines (due to "air bubbles" in the glass when being drawn into tubing), or other flaws.

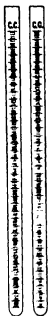


FIG. 4.  
Eggertz Tubes

These tubes may be used in a color camera (see page 15) or simply held at an angle of about  $45^\circ$  to a sheet of white paper with their lower ends touching it. The color matching is made in diffused sunlight or with a frosted electric lamp of filament type. See "Determination of Carbon in Steel," page 150.

**4. Julian Tubes.**—Figure 5. These tubes are similar to Eggertz tubes, except that they are bent at an obtuse angle at the top end and are not graduated over the lower portion. The bent upper end permits mixing of the contents of the tube without the use of a stopper or a mixing plunger. The tubes are graduated to tenths of a cubic centimeter. The same care as to uniformity of diameter, transparency, absence of flaws, etc., must be taken as in selecting tubing for Eggertz tubes. For detailed specifications recommended by Johnson<sup>1</sup> for tubes to be used in the estimation of carbon in steel see page 150.



FIG. 5.  
Julian Tubes

In comparing colors, Julian tubes may be used in a color camera or held against a sheet of white paper as directed above.

**5. Hehner Cylinders.**—Figure 6. Hehner cylinders are used in pairs and are the simplest form of apparatus employed in matching colors by the balancing method. Each cylinder has a glass stopcock about 2.5 cm. from the bottom through which liquid may be drawn off until the color in

<sup>1</sup> Chemical Analysis of Special Steels, Steel-Making Alloys, Their Ores and Graphites, p. 307. John Wiley & Sons, New York, 1920.

the two cylinders is the same in intensity when viewed vertically. The cylinders should have flat, carefully ground and polished bottoms of clear glass, and should be uniform in bore. They are graduated at 1 cc. intervals and should have a capacity of 100 cc.

Whitson<sup>2</sup> modifies the Hehner cylinder into a simple type of colorimeter. To the side delivery tube near the bottom he connects a sliding reservoir by means of a piece of rubber tubing of convenient length. This permits quickly changing the length of the standard colorimetric solution. The sample solution is placed in a cylinder of the same dimensions as the one containing the standard, but it need not have a side delivery tube. Both tubes are mounted in a suitably constructed camera. The use of a camera is not necessary unless the highest degree of accuracy is required.

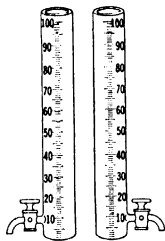


FIG. 6.—Hehner Cylinders

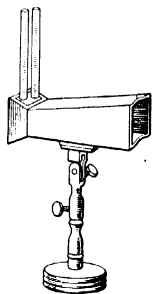


FIG. 7.—Color Camera

**6. Color Camera.**—Figure 7. The colorimetric color camera is essentially a long, narrow box, painted dull black inside, and carrying holders for two glass tubes near one end and having the other end shaped to fit the face of the observer so that no side light may enter. The end near the tubes is covered with a piece of ground glass so as to give a uniformly diffused light. A light blue glass is sometimes preferable to a white glass.

## COLORIMETERS

**7. Steiger Colorimeter.**—Figure 8. The construction and use of the Steiger Colorimeter is described by Steiger<sup>3</sup> as follows:

Instruments using the principle upon which this one is based—the ratio of the thickness of the liquid through, and not the actual dilution to equal concentrations—are not applicable to all colorimetric deter-

<sup>2</sup> Bull. 85, Wisc. Agr. Expt. Station; see also, Schreiner and Ferris, J. Am. Chem. Soc., 26, 961 (1904).

<sup>3</sup> J. Am. Chem. Soc., 30, 215 (1908); also Bulletin 700, U. S. Geological Survey, p. 37 (1919).



minations. It will be found, in comparing such a solution as is used in the colorimetric determination of manganese and some other substances, that there is a change not only of the intensity of the color, but also of the color itself, making it impossible to find a point at which two solutions of different concentrations will have the same depth of tint. In some other cases, as for instance, the yellow color of the higher titanium salts, this principle gives perfect satisfaction.

The instrument to be described consists of two wooden boxes, the interior portions of which are finished in dead black. In Fig. 8 (a),

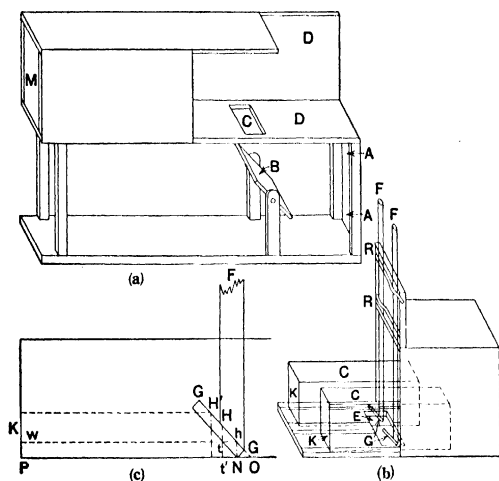


FIG. 8.—Steiger Colorimeter

*AA* is a piece of finely ground glass, and this should be illuminated with the full light of the sky. *B* is a mirror mounted to swing so that light may be thrown perpendicularly through the hole *C*.

The second portion of the apparatus consists of a box, as shown in Fig. 8 (b), made with two parallel grooves in the bottom, in which the two glass cells *CC* can be moved back and forth, and the hole *E*, which admits light reflected by the mirror *B* of Fig. 8 (a). These cells are about 15 cm. long, 2.5 cm. wide, and 5 cm. deep. On the bottom of each cell and near the outside edge is engraved a scale, a convenient unit for which is the millimeter. *FF* are glass tubes with

mirrors,  $GG$ , attached to the lower ends at an angle of  $45^\circ$ . These tubes may be lifted up when it is desired to remove the cells; they may also be removed entirely from the clips  $RR$  for cleaning purposes, but they should be pushed down when in use so that the lower edges of the mirrors touch the bottoms of the cells. When ready for use this box is placed in the space marked  $DD$ , Fig. 8 (*a*). Care should be taken to place the mirrors at an exact angle of  $45^\circ$ .

Under these conditions, in each cell, all light coming through the bottom of the cell and reflected through the end  $K$  will go through the same thickness of liquid, and if the mirror were a reflecting surface coming in direct contact with the liquid this distance would be represented by the line  $OP$ , Fig. 8 (*c*). There is a small error here, due to the converging of the rays to the eye; this is so slight, however, as not to cause any perceptible uneven illumination.

The mirrors being made of ordinary looking glass, the reflecting surface will be the upper side  $GHG$ , Fig. 8 (*c*), and the light must go through the glass of the mirror before striking the reflecting surface, and the same on leaving. The distance which the light travels through the glass of the mirror will be represented by twice the length of the hypotenuse of an isosceles right-angle triangle, the equal sides of which are each equal to the thickness of the glass, and must be deducted from the length  $OP$ .

A ray of light entering the glass at the point marked  $N$  will travel to  $h$  and then be reflected to  $l$ . From  $l$  to  $w$  it will go through the colorimetric solution, and this distance is therefore the length to be measured. A point,  $H$ , is marked on the mirror near the outer edge, so that it may be seen in the same line of vision as the scale on the bottom of the cell, and perpendicularly above  $l$ . In looking through the end  $K$ , this mark will be recorded at the point  $l'$  directly below it on the scale, and  $l'P$  being the same as  $lw$ , the distance desired can be read off. The position of the point  $H$  is determined by measuring off, on the back of the mirror, a distance from the lower edge equivalent to three times the thickness of the glass. It may be convenient, if thin looking glass has been used, to have this point farther up on the mirror ( $H'$ ), in order that it may be seen more plainly, but if so moved an addition must be made to the observed reading equivalent to one of the sides adjacent to the right angle of an isosceles right-angle triangle, the hypotenuse of which is equal to the distance this point has been removed from  $H$ . It is convenient in making the graduation

on the cell to allow for this correction. The reading can then be made directly.

Glass cells to answer the purpose may be had of any of the large supply houses, but not graduated; the graduation must be done in the laboratory. The supports *FF* can be made of rather heavy walled glass tubing, about 1 cm. outside diameter.

The mirrors are made of a good grade of looking glass, the lower and top edges blackened, and cemented to the ground ends of the glass tubes with Canada balsam, after which the backs are coated with paraffin. Paraffin answers well as a coating for a large number of colorimetric solutions. In case a liquid is to be used which attacks paraffin, a substitute must be employed which is unaffected by the liquid in question. It will be found necessary to replace the mirrors from time to time, as it is not possible so to protect the silvered surfaces as to prevent the gradual eating in from the edges by the various solutions used.

The comparison is made by pouring a solution of known strength into one of the cells. The unknown solution made up to a definite volume is put into the other. The left-hand cell is then placed at a convenient point, which should be determined by the depth of color of the solutions it contains. The right-hand cell is then moved back and forth till, on looking in the end *M* of the apparatus, Fig. 8 (*a*), the two mirrors appear to be of the same shade.

The strengths of the two colorimetric solutions being inversely proportional to the thickness of the liquids looked through, by substituting in the following equation the amount of the material to be determined may be found.

Let *R* equal the reading of the cell containing the known solution with a concentration *C*, and *r* the reading of the cell containing the unknown solution, which has a concentration *c*, then

$$c = \frac{RC}{r}$$

**8. Schreiner Colorimeter.**—Figure 9. The Schreiner Colorimeter<sup>4</sup> is essentially a pair of graduated glass tubes, one for the standard solution and the other for the sample solution, the heights of the columns of liquid in each being changed by means of two smaller immersion glass tubes. For convenience in making an accurate

<sup>4</sup> J. Am. Chem. Soc., **27**, 1192 (1905); also Bulletin **31**, U. S. Dept. of Agri., Bur. Soils.

color matching, a reflector is placed at the bottom to reflect the light up through the tubes, and a mirror placed at the top to receive the color image. The mirror is fastened into one end of a wooden box and is viewed by the observer at the opposite end. All the parts of the apparatus that come in contact with solutions are made of glass.

The immersion tubes *A* (Fig. 9) are 26 cm. long and about 2 cm. in diameter. The top ends of the tubes are mounted in wooden blocks which fit into grooves on the body of the colorimeter. This arrangement permits easy removal of the tubes for cleaning. Tubes *B* are also 26 cm. long but have a diameter of about 3 cm. These tubes are graduated into divisions 2 mm. apart and on the reverse side have marks at 50 cc. and 100 cc. for convenience in making solutions up to a definite volume. The *B* tubes are supported by a block of wood about midway of the camera and are held in position by brass springs or clamps, *C*. The tension of the clamps is adjusted so that the tubes may be moved freely up and down by hand and yet be firmly held in position when the setting is made. The bottoms of

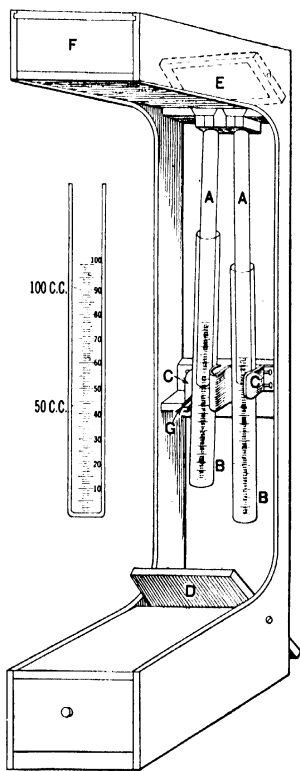


FIG. 9.—Schreiner Colorimeter

both the *A* tubes and the *B* tubes must be flat and the glass well ground and polished. The openings to the *A* tubes may be covered with a microscope slide to keep out dust. The reflector *D* carries a sheet of white cardboard or opal glass. From *D* the light passes up through the tubes, strikes the mirror *E*, and is reflected to the eye of the observer at *F*. The mirror should be of colorless

glass and without flaws. The approximate dimensions of the camera are  $70 \times 32 \times 16$  cm.

In carrying out a comparison the sample solution is diluted to a definite volume and poured into one of the tubes. The latter is then put in place in the camera and adjusted to a suitable height, say at the 50 scale division. The standard solution is poured into the other tube, the latter put in position in the camera and moved up and down until its image in the mirror is the same in intensity of color as that of the sample solution. The two solutions are then said to be balanced. The setting of the standard is read off the graduated tube by noting the scale division that coincides with the ground bottom of the immersion tube. This reading gives the height of the column of the standard solution which has the same intensity of color as the column of sample solution. It follows that the strengths of the two solutions are inversely proportional to the heights of the columns, i.e., to the scale readings. If we denote the reading of the standard solution by  $R$ , its strength by  $S$ , and denote the reading of the sample solution by  $r$  and its unknown strength by  $s$ , then

$$s = \frac{R}{r} S.$$

This formula, of course, also applies if the standard solution is fixed at a convenient reading and the sample solution moved up and down until the two color images have the same intensity.

It is sometimes more convenient (especially if the standard solution deteriorates fairly rapidly) to use standardized glass slides in place of a standard solution. The standardized colored glass slide is inserted at  $G$  below one of the immersion tubes in place of the standard solution tube. The immersion tube is retained so that the two images will be similar when viewed through the observation box at  $F$ . The constant  $C$  for the glass slide is obtained from the formula,

$$C = RS,$$

where  $R$  is the scale reading for the standard of strength  $S$ .

Simple colors can sometimes be used but usually a combination of colors is necessary to give the required tint of color. A series of slides of different intensities, but bearing a simple relation to each other, should be available. Such a series makes it possible to measure both

weak and strong solutions. The strength  $s$  of the sample solution, when measured against a standard slide, is obtained by the formula

$$s = \frac{C}{r},$$

where  $C$  is the constant above mentioned and  $r$  is the scale reading of the sample solution.

NOTE.—The specifications of the U. S. Bureau of Soils for the glass parts of the Schreiner colorimeter are as follows: "The measuring colorimeter tubes are to be 26 cm. long, with *inside* diameter as nearly as practicable 27 mm.; in no case less than 25 mm., and not greater than 29 mm. The glass is to be colorless and the bottoms well ground and polished, with the internal surfaces of the bottoms plane, in no case appreciably convex or concave; the bottoms to be ground down sufficiently so as to make the ground surfaces a little larger than the internal diameters of the tubes, each of the tubes to be provided with an etched scale of 100 two-millimeter divisions. The scale is to begin at the level of the inner surface at the bottom, the length of the marks being 6 mm., and every fifth mark 12 mm., numbering every tenth mark, on the right side, 10, 20, 30, etc., beginning at the bottom. On the reverse side of each measuring tube there are to be etched two capacity marks, one for 50 cc., and the other for 100 cc. The smaller tubes are likewise 26 cm. long, with *outside* diameter not greater than 20 mm., and not less than 18 mm., with the thickness of the glass the same in all the tubes. The bottoms of these tubes are to be carefully ground and polished and the inside of the bottom never sensibly convex or concave. They must be of colorless glass with the bottoms so ground that the diameter of the ground portion exceeds the internal diameter of the tube. These tubes are to be provided with neither scale nor capacity marks."<sup>5</sup>

**9. Kennicott-Campbell-Hurley Colorimeter.**—Figures 10 and 11. Like the Schreiner Colorimeter, the Kennicott-Campbell-Hurley<sup>6</sup> is a colorimeter for general use, is simple in construction and operation, and gives accurate readings. Essentially this colorimeter consists of two glass comparison tubes, one of which is attached by means of a glass tube to a glass cylindrical reservoir carrying a glass plunger.

<sup>5</sup> Schreiner, *loc. cit.*

<sup>6</sup> Kennicott and Sargent, *Chem. Engineer*, **5**, 213 (1906-07); Campbell and Hurley, *J. Am. Chem. Soc.*, **33**, 1112 (1911); *cf. Smeaton, ibid.*, **28**, 1433 (1906).

By lowering or raising the plunger the level of the liquid in the tube is adjusted until its color is the same in intensity as that of the liquid in the other tube. The colors are matched by observing their images in two mirrors arranged so that the observer sees a single circular field divided vertically, one-half the field coming from one tube and the other half from the other tube. (See Fig. 10.) When the intensities

of the two halves are the same, the dividing line is almost imperceptible.

Figure 11 shows diagrammatically the essential features of construction of the Kennicott-Sargent colorimeter as modified by Campbell and Hurley. The following is their description of the apparatus:<sup>7</sup>

The unknown solution is placed in the left-hand tube *A* which is 19 cm. long, 3 cm. in diameter, and graduated for 15 cm. The standard solution is placed in the right-hand tube *B* which is the same size as *A*, the graduated portion being divided into 100 divisions of 1.5 mm. each. The tube *B* is perma-

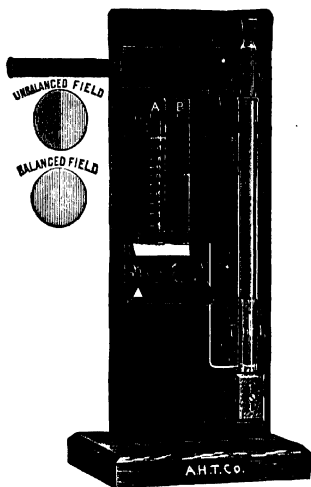


FIG. 10.—Kennicott-Campbell-Hurley Colorimeter

nently connected by a glass tube with the reservoir *C* in which the glass plunger *D* works, so that the level of the liquid in *B* can be readily controlled by raising or lowering the plunger. As the tube *B* and reservoir *C* are made in one piece, the liquid used for the standard solution comes in contact with glass only, thus preventing any possibility of chemical change due to contact with the container. The plunger is provided with a rubber collar, *E*, so placed as to prevent the plunger from accidentally striking and breaking the bottom of the reservoir. The tubes *A* and *B*, with the connecting reservoir, rest on wooden supports, the one under *A* and *B* being provided with holes for the passage of the light, and are held in position by spring clips, *FF*. This arrangement allows the glass parts to be

<sup>7</sup> *Loc. cit.*

readily removed for cleaning and filling. The light for illuminating the solution is reflected upward through the tubes *A* and *B* by means of the adjustable mirror *G*. The best results are obtained by facing the colorimeter toward a north window in order to get reflected sky-

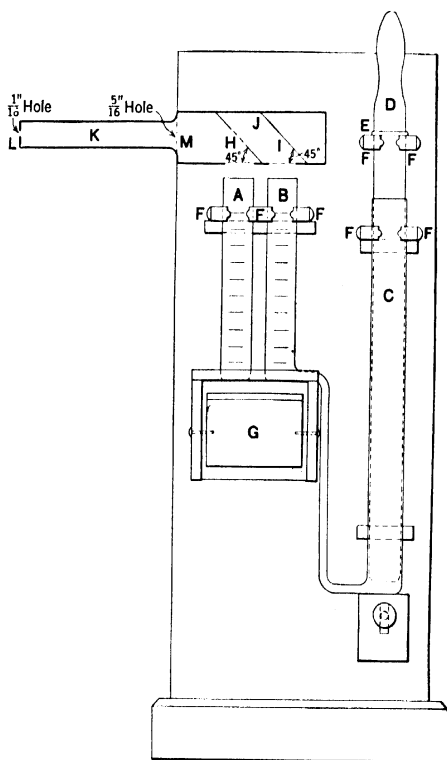


FIG. 11.—Kennicott-Campbell-Hurley Colorimeter?

light through the tubes, care being taken to avoid light reflected from adjacent objects. The black wooden back of the colorimeter serves the double purpose of a support for the parts of the instrument and of a screen, as it is interposed between the color tubes and the source of light.

The light passing upward through the tubes *A* and *B* impinges on



the two mirrors, *H* and *I*, cemented to brass plates sliding in grooves cut at an angle of  $45^\circ$  in the sides of the wooden box, *J*. This box is supplied with a loose-fitting cover, thus allowing easy access for the purpose of removing and cleaning the mirrors. The mirror, *II*, is cut vertically and cemented in such a position as to reflect one-half of the circular field of light coming through the tube *A*. The light passing upward through *B* is reflected horizontally by the mirror *I*, through a hole in the brass plate supporting the mirror *II*. One-half of the circular field of light from the tube *B* is cut off by the mirror *II*, the vertical edge of which acts as a dividing line between the two halves of the circular field. The image of one-half of the tube *B* is then observed in juxtaposition to the opposite half of the image of the tube *A*.

The juxtaposed images are observed through a tube, *K*, 2.5 cm. in diameter and 16 cm. long, lined with black felt and provided with an eyepiece having a hole 1.5 mm. in diameter. At the point *M* in the tube *K* is placed a diaphragm having an aperture 8 mm. in diameter. All parts inside the box *J* except the mirrors are painted black so that no light except that coming through the tubes *A* and *B* passes through the tube *K*. By having the apertures in the eyepiece and diaphragm properly proportioned only the image of the bottoms of the tubes *A* and *B* can be seen, thus preventing interference of light reflected from the vertical sides of the tubes *A* and *B*.

A person looking through the eyepiece observes a single circular field divided vertically by an almost imperceptible line when the two solutions are of the same intensity. By manipulating the plunger *D*, the level of the liquid in *B* can be easily raised or lowered, thus causing the right half of the image to assume a darker or lighter shade at will. In matching colors with an ascending column in *B*, that is, gradually deepening the color of the right half of the field, the usual tendency is to stop a little below the true reading while in a comparison with a descending column the opposite is the case. In making a comparison, therefore, several readings with an ascending column and several with a descending column should be made and an average of all readings taken.

**10. Nessler Tube Colorimeter.**<sup>8</sup>—Figures 12 and 13. This colorimeter is similar to the Kennicott-Campbell-Hurley Colorimeter, a series of Nessler tubes and a rack replacing the stand carrying the two comparison cylinders and reservoir tube in the latter appara-

<sup>8</sup> J. H. Yoe, Ind. Eng. Chem. **19**, 1131 (1927).

tus. The light (north sky) is reflected by a small mirror placed on the rack at an angle, just below the "unknown" and comparison tubes. The mirror serves better than the white glass plate reflector of the Nessler rack when the sky is the source of light but not when a colorimeter lamp is used. The light passes upward through the Nessler tubes, impinges on the two mirrors *A* and *B* (Fig. 13) which are fastened to the wooden box at an angle of  $45^\circ$ , and is reflected horizontally through the metal observation tube. One-half of the circular field of light from the right-hand tube is cut off by mirror *A*, the vertical edge of which serves as a dividing line between the two halves of the cir-

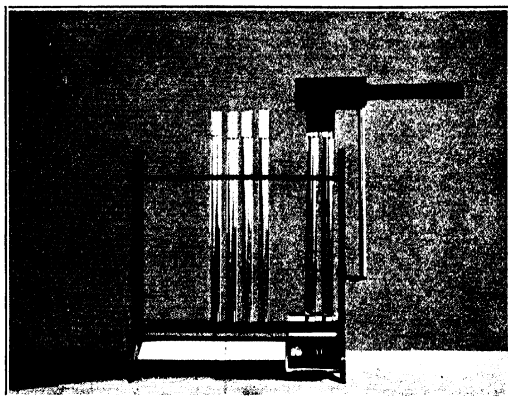


FIG. 12.—Nessler Tube Colorimeter

cular field. The image of one-half of the right-hand tube is then observed in juxtaposition to the opposite half of the image of the left-hand tube. The juxtaposed images are observed through a thin metal tube, 170 mm. long and 25 mm. in diameter, painted dull black inside and out and provided with an eyepiece having a hole 1.5 mm. in diameter. At the other end of the tube is a diaphragm having an aperture 8 mm. in diameter. By having the apertures in the eyepiece and diaphragm properly proportioned, only the image of the bottoms of the Nessler tubes can be seen, thus preventing interference of light reflected from the vertical sides of the tubes. Upon looking through the eyepiece the observer sees a single circular field, divided by an almost imperceptible line when the two solutions have the same intensity.

The colorimeter is attached to the Nessler rack by means of a metal tube support which slides snugly down over a vertical rod securely fastened to the rack. By this arrangement the colorimeter may be quickly and easily raised and turned on its horizontal axis, thus permitting interchanging the Nessler tubes in the series of standard solutions until a match with the "unknown" is obtained. In practice, the *approximate* match is first obtained in the usual way by looking down vertically through the tubes in the rack and the *final* match made by swinging the colorimeter into place.

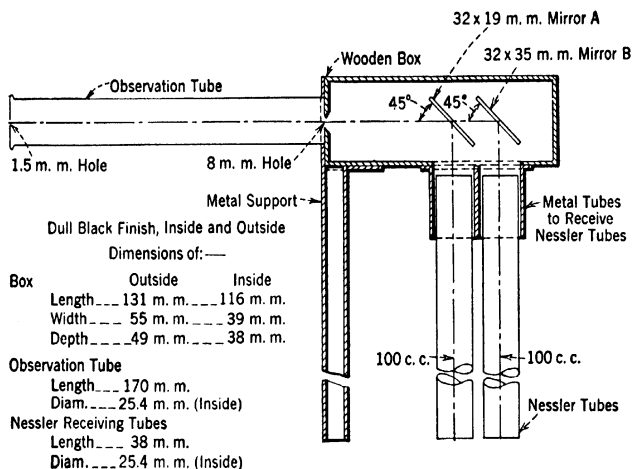


FIG. 13.—Nessler Tube Colorimeter

The colorimeter box is painted dull black inside and out. It is fitted with a removable cover, which permits easy access to the mirrors for the purpose of adjusting and cleaning.

A screen (not shown in the figures) made of a piece of stiff cardboard and painted dull black may be interposed between the Nessler tubes and the source of light. The use of a colorimeter lamp is helpful and is recommended if the highest precision of matching is required.

**11. Bock-Benedict Colorimeter (Improved Model).**<sup>9</sup>—Figure 14. The Bock-Benedict is a new form of colorimeter of simple construction and moderate price. It was designed particularly for biochemistry. The instrument is light and, at the same time, is well balanced. Its

<sup>9</sup> J. Biol. Chem., **35**, 227 (1918). Fully describes the first model.

large spreading base makes accidental upsetting very difficult. It yields the same light field and the sharp color change of the Duboscq instrument and its readings are as rapidly and as accurately made.

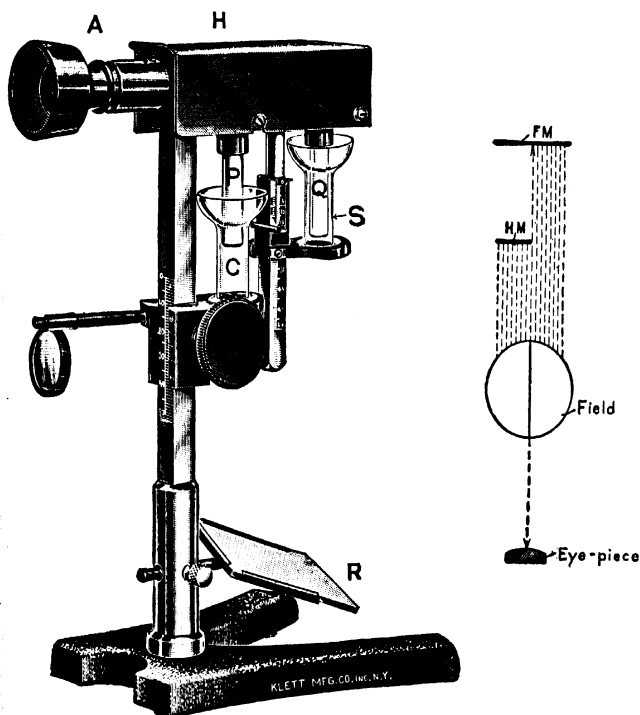


FIG. 14.—Bock-Benedict Colorimeter

In the Bock-Benedict colorimeter the expensive pair of parallelopipeds forming the main part of the optical system in the Duboscq colorimeters is replaced by two mirrors, *HM* and *FM*. One of these mirrors (*HM*) reflects the light from the unknown solution, and the other (*FM*) the light from the standard solution, into the observation tube *A*. The illuminating mirror *R* is made by plating finely ground glass. Such a mirror prevents direct reflection of objects being visible by the reflecting mirrors *FM* and *HM*. The illuminating mirror *R*

throws the light into the reflecting mirrors *FM* and *HM*. Mirror *HM* is so arranged as to take up half (left) of the field of vision in the observation tube *A* and is placed over the plunger *P*, giving a reflection of color in the unknown solution which is contained in the movable cup *C*. Mirror *FM* is arranged so as to take up the other half (right) of the field of vision in the observation tube *A* and is placed over the plunger *Q*, giving a reflection of color in the standard solution which is contained in the movable cup *S*. The top piece of the apparatus containing mirrors *FM* and *HM* is covered by a removable housing, *H*, which protects the mirrors from corrosion.

Before using the colorimeter, or a new cup, it is always advisable to check the instrument to insure accuracy. This is carried out as follows: The cup is run up until its bottom and the bottom of the plunger meet. See if the vernier on the scales reads *zero*. If not, run the cup down again and loosen the set screw. Pull the plunger down a millimeter or two and then run the cup slowly up again, while closely observing the vernier and scale. As soon as the vernier has met the *zero-point*, tighten the set screw of the plunger and the instrument is properly set.

In order to use the colorimeter, put both cups in place and raise them until each just meets the bottom of its plunger. Then look through the observation tube *A* and move the large reflector *R* until both halves of the field are exactly equal. Now put the standard solution in cup *S* and the unknown solution in cup *C*. Set cup *C* at the desired height and move cup *S* slowly up and down by means of pinion wheel until both halves of the field appear the same. Read the scale. Make several readings and take the average. A magnifying lens attached in front of the scale assists in making a rapid and accurate reading. All readings must be made with the housing *H* in place.

**12. Kober Colorimeter.**<sup>10</sup>—Until the World War, the only accurate instrument of the plunger type was that of the French Dubosq make. The supply of these in the United States was soon exhausted and the War prevented further importations. Kober, who in 1912 was the first to show that nephelometry was a means of analysis and similar to colorimetry, found that the plunger type of colorimeter with a few accessories made an excellent nephelometer, but, owing to the scarcity of Dubosq instruments, was forced to enter into the manufacture of plunger instruments in order to supply the demand in the

<sup>10</sup> J. Biol. Chem., **29**, 155 (1917); *ibid.*, **47**, 19 (1921).

United States. In spite of difficulties—two larger optical instrument manufacturers refused coöperation; two smaller companies proved incompetent—Kober had developed the manufacture of his instrument so that an ample supply of instruments was available for the U. S. Army, when the United States entered the War in 1917.

Kober succeeded in improving the plunger type in several respects. He considered the colorimeter (also nephelometer) as a light balance, which he found theoretically, and also in actual practice, was like the most sensitive gravimetric balances in that it was impossible to keep both sides in perfect equilibrium without frequent adjustment. Mechanical disturbance, temperature, dust, light distribution, personal variations in the observer, etc., all served to make the unadjustable Duboscq instrument less accurate. To make these adjustments convenient, Kober in his instrument provided adjustable scales and verniers, and adjustable or so-called split reflectors. In addition to these improvements a hollow glass plunger was substituted for the solid glass plunger of the Duboscq, as the latter showed appreciable absorption of light. Mechanical improvement resulted in putting the lowering and raising mechanism behind the instrument to prevent sources of error due to stray reflections. Recognizing that in the use of optical instruments, the convenience of the operator and sparing him useless or unnecessary efforts, both mechanical and optical, would result in greater accuracy, especially in repeated and routine determinations, Kober introduced into his instrument the so-called "top" reader,<sup>11</sup> the use of a double-milled head for rough and fine adjustment, and the elimination of glare which, falling upon the observer's eyes while he is using the instrument, causes destruction of eye-sensitivity.

One of the Kober instruments, which embodies these improvements, is described as follows: (1) The milled heads formerly at the top of the instrument, are placed at the bottom, which allows the hands to rest on the table or other support and the adjustments to be made with the fingers (shown in Fig. 15). (2) An auxiliary scale is provided at the top of the instrument, consisting of the following parts: two scales engraved upon the side away from the operator, fastened to the movable stages, so that when the stage is being moved up or down, the scales move with it; a stationary vernier, protruding beyond the top plate, also engraved upon the side away from the operator, fastened to the top of the instrument. A mirror facing the operator at

<sup>11</sup> Made with the assistance of Robert E. Klett.

an angle of 45 degrees is placed in front of the protruding scale and vernier, so that an image of the two is reflected vertically. A magnifying glass of the same focal length as the telescope, serving as a second eyepiece, has been placed close beside the regular eyepiece, directly

above the mirror, showing the image of the scale enlarged in good light.

Figure 15 shows the entire instrument without the lamp house.

In Fig. 16 are shown the fields that are observed through the two eyepieces. The ease of reading the scale is apparent.

In Figure 17 is shown the construction of the auxiliary scale. This auxiliary scale is engraved to 60 mm. but with the vernier is readable to only 50 mm., which is ample for most work. If heights above 50 mm. are to be measured, the original vernier can be used. The setting of the zero-point is easily and accurately accomplished with a micrometer arrangement, as may be seen at *A*, by a milled head working against a spring. This convenient method of zero-point adjustment, together with the very simple method of using the instrument, the method of Lamb, Carleton and Meldrum (see Vol. II), where the height of the standard solution (*S*) is kept constant, makes the operation of the instrument and calculation of results extremely

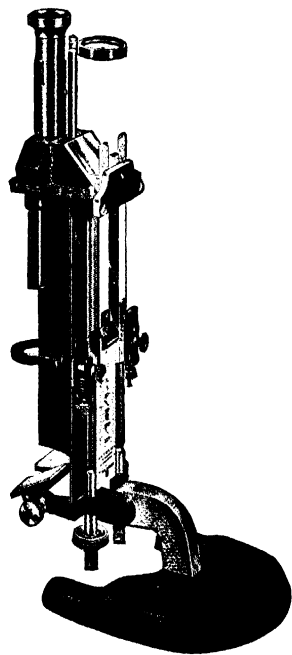


FIG. 15.—View of the Kober instrument (1921) without the lamp house, showing (a) the two eyepieces, the scale, and the mirror at the top of the instrument; (b) the micrometer adjustment of the zero-point; and (c) the milled heads operating the cups at the bottom of the instrument.

simple and easy, without, however, sacrificing accuracy or deviating from the fundamental basis of either colorimetry or nephelometry.

In Fig. 18 is shown the instrument attached to a lamp house.

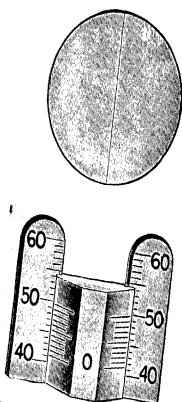


FIG. 16.—The two fields as seen through the two eyepieces. The upper figure shows the two semi-circular fields; the lower figure shows the stationary vernier and the two adjustable scales.

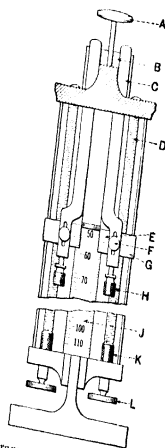


FIG. 17.—Diagrammatic sketch of the Kober instrument (1921). *A*, magnifying lens for the horizontal scale; *B*, mirror at an angle of  $45^\circ$ ; *C*, movable to 100 mm. scale; *D*, screw-threaded rod; *E*, vernier for 50 zero adjustment; *F*, lock-nut for the zero adjustment; *G*, movable cup carrier; *H*, micrometer for *K*, scale from 50 to 100 mm.; *J*, scale from 50 to 100 mm.; *K*, knurled thumb-screw for rapid movement; *L*, knurled thumb-screw for fine adjustment.

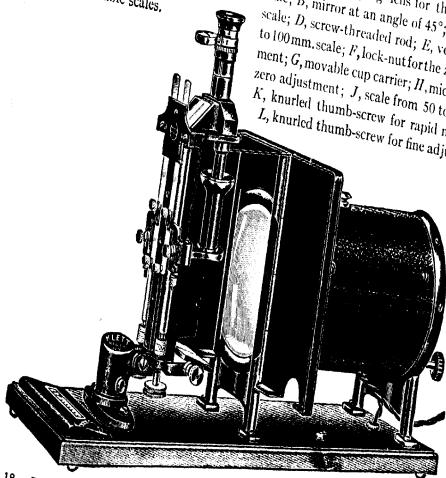


FIG. 18.—The Kober colorimeter (1921) and lamp house showing the split reflectors as well as the front of the instrument illuminated by the light from the lamp house.



**13. Kober Precision Colorimeter-Nephelometer.**<sup>12</sup>—This instrument is designed for routine and scientific routine precision work. The lowering and raising of the cups is accomplished by means of a hydraulic system, which permits the use of an automatic scale reader.

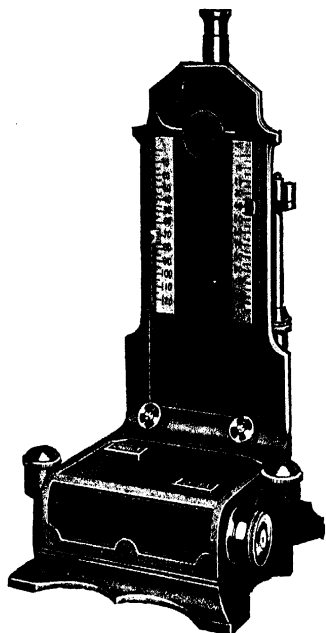


FIG. 19.—Kober Precision Colorimeter-Nephelometer. View of back of instrument. Shows the knurled knobs on the side of the base for the vertical movement of the cups. Also knobs on the top of the base for adjusting the reflectors. Vertical millimeter scales and verniers on the back of the instrument and automatic scale readers on the top of the base are shown roughly.

This reader gives the millimeter and fractions of a millimeter directly in figures, eliminating the reading of the verniers for routine and other repeated measurements. This approximately decreases the amount of eye work by fifty per cent.

The usual vertical millimeter scale and verniers are also provided for the purpose of standardizing, and checking the automatic reader. The construction and mechanism are such that maximum solidity and freedom of vibration is obtained. The knobs regulating the mirrors are placed in the back of the instrument so that the accidental displacement of the mirrors while making the measurements is made practically impossible.

The plungers and cups in this instrument are separated by a minimum of horizontal distance, requiring therefore for either colorimetry or nephelometry a very small cone of light.

**14. Duboscq Colorimeter. Bausch & Lomb Model.**—The frame, A (see Fig. 21), of the instrument, consisting of base and upright, to

<sup>12</sup>The complete details of this instrument will be published in a future scientific journal article. The instrument and all accessories are manufactured by the Precision Scientific Company, Chicago, Ill.

which are attached the various components of the optical system, is made of heavy iron castings, so constructed as to provide for stability and permanent alignment of the optics.

The mirror, *B*, which is adjustable about a horizontal axis, is provided with two reflecting surfaces, one a plane silvered mirror, and the other a plate of fine ground opal glass which reflects light diffusely. These plates are cemented in a metal frame by means of a specially prepared acid- and alkali-proof composition, so that liquids which are accidentally spilled on the mirror cannot penetrate to, and cause deterioration of, its silvered surface. The single mirror of suitable size assures equal intensity of light for both tubes.

The two movable cups, *C* and *C'*, for holding the liquids under examination, consist of thick-walled cylinders of glass. These cylinders fit into tubes of brass, threaded at the lower end so that, by means of a metal screw cap, a plane glass plate may be forced firmly against the glass cylinders, whose ends are finely ground, in such a manner as to secure a water-tight joint. A rubber washer is placed between the glass plate and the shoulder of the metal screw cap for the purpose of equalizing the pressure and preventing the possibility of breakage. The plate, which forms the bottom of the cup, is made of optical glass having plane parallel surfaces. The metal sleeves, which surround the glass cylinders, are slotted so that the height of the liquid in the cups may be observed readily. All parts of the cups are interchangeable.

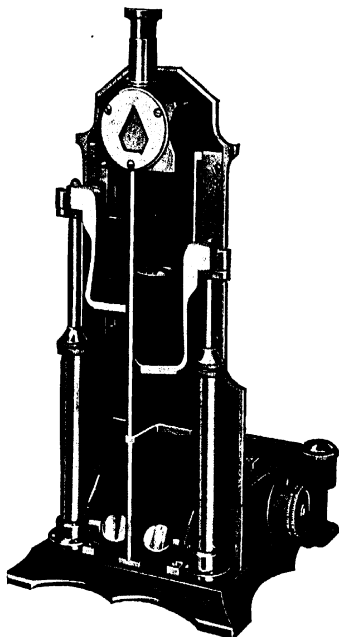


FIG. 20.—Kober Precision Colorimeter-Nephelometer. View of front of instrument. Shows the adjustable "split" mirrors, movable platforms and cups, hollow plungers, prism house and eyepiece.

able. The tube may conveniently be shaded by turning the single slot to the rear.

The design of these cups is the result of suggestions made by Dr. Folin, of the Harvard Medical School. He has also contributed other valuable ideas in the present improved model of this instrument. The caps can be thoroughly and easily cleaned, and in the event of breakage of glass parts replacements can be made without inconvenience or delay, as extra glass cylinders and plates may be secured for such an emergency.

The cups may be raised and lowered by turning the milled heads,  $D$  and  $D'$ , each of which actuates a rack and pinion. The slides, along which the motion takes place, insure easy and accurate settings. The pinions and operating heads are always in a fixed location, so that the observer's readings are controlled only by observation and not by the position of the pinion heads, thus tending to eliminate the personal equation in this detail.

Two solid glass plungers of optical glass, matched for color and having optically plane and parallel ends, are attached to the frame of the instrument by means of metal adapters into which they are firmly cemented. They are located in a fixed position in the axis of the instrument, which passes through the centers of the cups. Any injury to cups or plungers, which might be caused by bringing them violently together, is prevented by means of two adjustable stops, which determine the highest position to which the movable cups can be raised.

The scales  $S$  and  $S'$  (see Fig. 21) are etched on glass and read by transmitted light coming from the source of illumination of the colorimeter. A right-angle prism mounted in the back of the stand reflects the light from scales into a vertical path so that a very slight movement of the head from the eyepoint position brings the scale and vernier into view. A lens mounted above the prism assists by magnifying the scale and vernier so that reading is easily accomplished from the eyepoint. With the plunger and cups in contact the vernier is adjusted to read zero by means of a thumb screw on the back,  $K$  (Fig. 22).

The eyepiece or coincidence prism, which is located between the plungers and the observing microscope, consists of two rhomboids. The function of this prism is to bring to a common center, or axis, the light which passes through the two sets of cups and plungers, so that the intensities of the two beams may be observed in two adja-

cent fields with one eye—a condition essential for accurate comparison or matching of intensities.

The prism box and connections are all constructed with the idea of excluding dust, even to omitting the usual opening at the side for removal of prism. A permanent setting of the prism is accomplished, while the entire prism box is removable through having screw

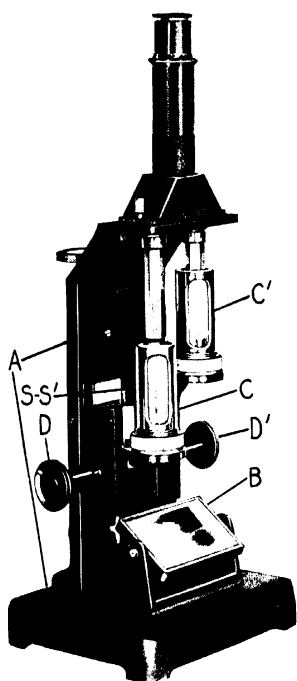


FIG. 21.—B. & L. Duboscq Colorimeter.

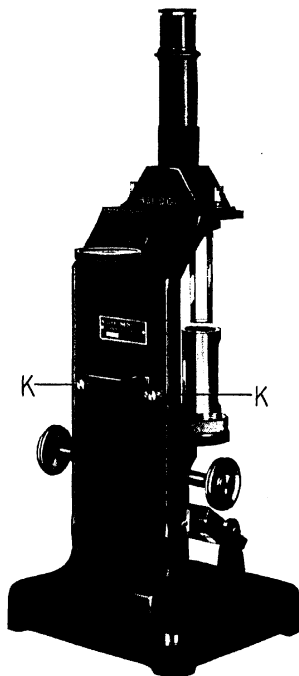


FIG. 22.—B. & L. Duboscq Colorimeter.

heads with finger grip to hold it in place on its supporting plate. In this way the specific parts of the prism which might require cleaning can be exposed for such attention.

The dividing line separating the halves of the field of the instrument as viewed through the observing microscope, is formed by adjacent ends of the rhombohedral prism, on which two

narrow surfaces have been polished in order to obtain close contact and a thin smooth line. The field of view of the observing microscope appears as a circle, divided into halves by the dividing line. On account of the inversion caused by this microscope, the light which illuminates the right semi-circle thus formed has passed through the left beaker, plunger and prism, while that illuminating the left semi-circle has passed in a like manner through the right beaker, plunger and prism. In this manner is formed what is essentially a photometric field, by means of which comparisons of intensities may be accurately made, the principle of the instrument being based on the adjustment of the depth of the liquids in the cups, so that the appearance of the two adjacent halves of the field is identical. In other words, when the two halves are matched, the concentrations of the two solutions in the beakers are inversely proportional to the depth, read directly from scales in the rear of the instrument, of the liquids between the lower surface of the plungers and the bottom of the cups.

The observing microscope consists of an eyepiece and an erecting system. The purpose of the erecting system is to permit the introduction of diaphragms to act as limiting apertures, by means of which a correct passage of light rays through the instrument, an evenly illuminated field, and an absence of disturbing reflections are brought about.

A sliding cover is provided for the purpose of shutting off all extraneous light which might in some cases affect the setting. It also serves to protect the cups and plungers when the instrument is not in use.

To test the adjustment of the instrument make sure that the bottoms of the cups are in contact with the lower ends of the plungers at the zero position of the scales. If this condition has not been fulfilled, make the necessary adjustment of the stops, as well as of the verniers. It is to be noted in this connection that the position of the bottom of the cylinder with respect to the lower surface of the plunger may be slightly varied by tightening or loosening the screw cap which forces the rubber washer against it.

Care should be exercised not to scratch the lower surface of the plunger by rubbing the cover glass against it under pressure. In order to obviate all danger of damage to the surfaces of both, it is advisable to place a thin piece of tissue paper between them while this adjustment is being made.

Rack the cups,  $C$  and  $C'$ , down to their lowest position, and remove them from the instrument, taking care that they do not strike the glass plungers.

Turn the entire instrument toward an even, ample source of light, preferably diffuse daylight, or very white artificial light, and, while looking through the eyepiece, adjust the instrument and the mirror so that the two halves of the field appear of the same color and of equal brightness.

Pour a small quantity of "known" solution into one of the cups, and into the other cup some of the "unknown" solution. The cups should be not more than half filled with liquid, to avoid the danger of forcing some of the liquid over the top of the cup and causing it to spill on to the mirror or into the working parts of the instrument. If it should appear desirable to use a greater depth of liquid on account of faint coloration, care should be taken not to force liquid over the top of the cup.

Replace the cups in the instrument, and raise them by means of the racks and pinions until the lower ends of the glass plungers are well immersed within the liquids. Air bubbles, which in many instances are formed under the ends of the plunger, may easily be removed by slightly tilting the instrument. Now, while looking through the eyepiece, adjust the heights of the cups until an approximate balance is obtained on both sides of the field. It is now convenient to set the "unknown" liquid so that its depth, as shown on the scale,  $S'$ , is an exact number of millimeters, and then to raise or lower the "known" liquid until a match is secured, i.e., when both halves of the field appear equally bright and identical in color. Readings are now taken by means of the vernier on the scale,  $S$ , and recorded. To secure results of the greatest accuracy it is necessary to take the average of a number of independent readings.

When the two halves of the field are of the same brightness, *the color intensities of the two solutions are inversely proportional to the depth of the columns of the two solutions*. Let  $C_1$  = color intensity of the known solution;  $D_1$  = depth of the known liquid, as read on the scale,  $S$ ;  $C_2$  = color intensity of the unknown solution;  $D_2$  = depth of the unknown solution.

Then

$$C_2 \times D_2 = C_1 \times D_1,$$

and

$$C_2 = \frac{C_1 \times D_1}{D_2}.$$

The Bausch & Lomb Duboscq Colorimeter is made in two sizes, one permitting the examination of a column of liquid 50 mm. deep, the other a column 100 mm. deep. The latter is best adapted for

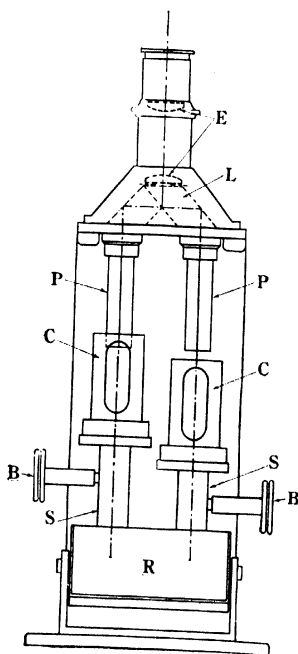


FIG. 23.—(Front view.)

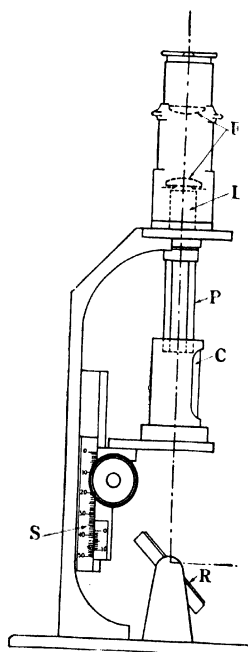


FIG. 24.—(Side view.)

Bausch & Lomb-Duboscq Colorimeter. *E*, eyepiece lenses; *L*, comparison prism; *PP*, plungers; *CC*, cups; *BB*, pinion buttons; *SS*, scales; *R*, reflector.

the measurement of liquids having a faint coloration. In all other respects the instruments are essentially identical.

Figures 23 and 24 show the instrument diagrammatically.

**15. Duboscq Small or Biological Colorimeter. Bausch & Lomb Models.**—The design of this instrument departs somewhat in external

appearance from the standard Duboscq type. The principle from the optical standpoint, however, is the same as that of the Duboscq.

The observing microscope consists of a collective and an eye lens, which do not form an exit pupil, so that a diaphragm near the eye-point is necessary to secure an evenly illuminated field and to cut off reflections.

The eyepiece or coincidence prism, the plungers and cups are of the same type as those used on the Duboscq Colorimeter. Light is

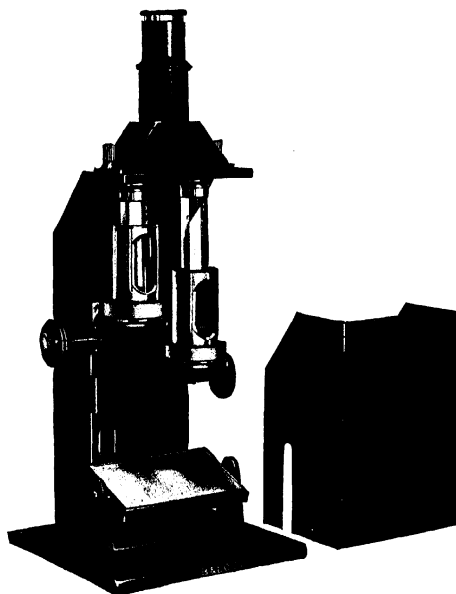


FIG. 25.—Bausch & Lomb Biological Colorimeter. Showing instrument with both cups adjustable by rack and pinion.

reflected into the instrument by means of a second surface mirror, whose upper face is finely ground. Illumination of a semi-diffuse character is obtained by its use. The mirror is cemented in a metal frame, adjustable about a horizontal axis, by means of an acid- and alkali-proof composition, which affords protection against the corrosive action, on the silvered surface, of liquids spilled on it.

Figure 25 shows the instrument with both cups adjustable by rack



and pinion. Fig. 26 shows the Biological Colorimeter with a special rotary drum for direct-scale reading. The rotary drum operates the right-hand cup. The scale on the drum is graduated in tenth millimeters, making reading from the eyepiece position easy and accurate. The index pointer follows the scale groove, thus eliminating any possible error in reading.

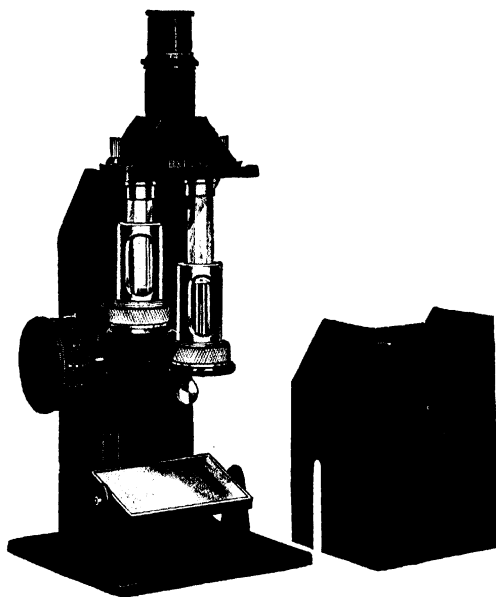


FIG. 26.—Bausch & Lomb Biological Colorimeter. With special rotary drum for direct scale reading.

**16. Duboscq Colorimeter. E. Leitz Model.**—This instrument (made in two sizes) is shown in Fig. 27 and its optical system illustrated diagrammatically in Fig. 28.

The rays, which should be derived from a uniform source of light, are reflected through two openings in the base of the apparatus, a plane mirror being used in the case of daylight, and a filament lamp with opal glass bulb in conjunction with a ground glass plate in the case of artificial light. The two pencils so admitted traverse two

identically similar cups and plungers placed 25 mm. apart, and are then made to approach one another in passing through the symmetrical Albrecht-Hüfner glass body, so that in the field of view they appear separated by a very fine line. When the observer focuses the Ramsden eyepiece above the glass body upon its upper edge he sees the field of view divided into two semi-circular half-fields. Of these the one on the right is uniformly filled with the light supplied

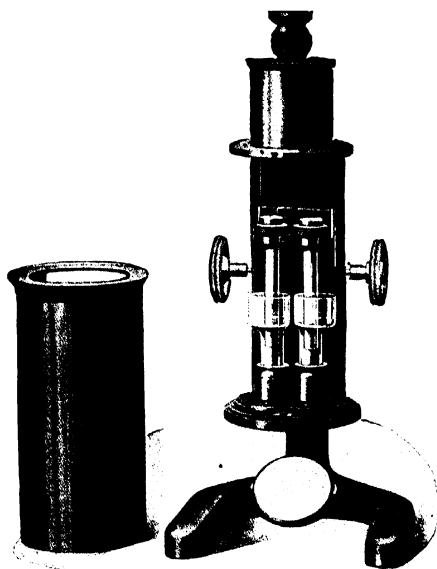


FIG. 27.—Leitz-Duboscq Colorimeter.

by the left pencil, whilst the field under the left eye is lit up by the right pencil. The two fields, being thus separated by an extremely fine line, can be compared with ease and accuracy. It is imperative, if an exact reading is to result, that the illumination should be absolutely identical on both sides. It is to be noted that any soiled patches on the plane mirror or the opal plate cannot fail to vitiate this condition.

The cups which serve to receive the solutions are mounted movably on slides. They are in their proper central position when they meet the limit stops at the rear. The plungers by means of which the color

identity is established in the half-field, can be displaced with the aid of rack and pinion motions, by means of which the required depth of strata can be set to a nicety. They are readily removable for the purpose of cleaning them, all that is necessary being to release the screw unions by which they are attached to the carrier. No force should ever be used when screwing them together. The space containing the cups and plungers is rendered light-proof by a slider,

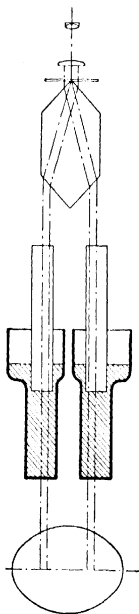


FIG. 28.

which excludes the access of adventitious light. In addition, the space which contains the Albrecht-Hüfner prism, and from which projects the Ramsden eyepiece only, is enclosed within a screw-on cylinder. The latter can be removed when it is required to clean the prism. The depths of the strata which result from the descent and ascent of the plungers can be read off accurately to 0.1 mm. by means of the scales and verniers above the milled heads. The reading of the scales is greatly facilitated by the prisms attached to the verniers.

After taking the readings the cups and plungers should at once be removed and cleaned. They should then be replaced and the apparatus closed up.

#### 17. Duboscq Colorimeter. Spencer Model.

Figure 29. This colorimeter is constructed on the Duboscq principle, though so many refinements have been added to the design that the prototype is hardly comparable with the present development. The cups are of one-piece construction, the bottom being fused directly to the side walls. The plungers are of optical glass carefully matched for absorption. Each cup is fastened on its pedestal by a very simple locking device so that it cannot become dislodged should the instrument accidentally be upset. Both cups are adjustable by independent rack and pinion adjustments. The depths of the fluids can be measured to 0.1 mm. The prism construction is such that the dividing line, as viewed through the eyepiece, is clear cut and sharp. This results in considerably greater accuracy. By means of reflecting mirrors, not shown in

the illustration, the depths of the fluids may be measured from the eyepiece position.

**18. Schmidt & Haensch Plunger Colorimeter No. 3a. Duboscq System.**—Figure 30. The plunger colorimeter is built symmetrically

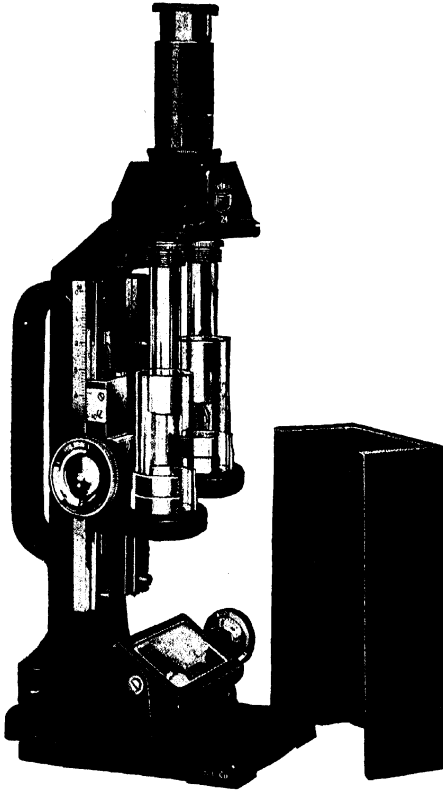


FIG. 29.—Spencer-Duboscq Colorimeter.

as far as the two sides are concerned, in regard to both optical and mechanical parts. The path of light from a photometric point of view is without criticism.

The height of the layer of liquid on both sides is 60 mm. and the

amount of liquid for this height is about 9.3 cc. for each cup. The exact height of liquid is read off with a vernier to 0.1 mm.

If desired, cells can be furnished for standard solutions with a fixed and definite height (50 or 25 mm.) having a screw cover. These cells can be put directly in the light path in place of one of the usual cups. To restore optical symmetry, both plungers are retained and

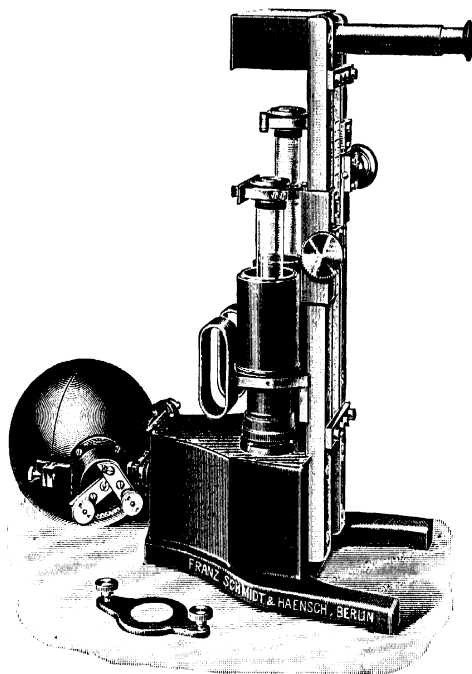


FIG. 30.—Schmidt & Haensch Plunger Colorimeter No. 34.

a glass is placed on the unknown side to compensate for the cover glass of the cell.

The field of vision is in two parts, with a line of separation so small as to appear almost vanished. The field appears to the eye to have a diameter of 18 mm. The illumination of both halves is effected from the reflection of a milk-glass plate always in the same position, so that equal illumination of both sides is obtained. The illumination of

the milk-glass plate is obtained from a light source consisting of an Osram-Opal Lamp placed directly in front of the plate or by means of an illumination "sphere."

To observe the light through filters of complementary colors, a revolving disk with light filters can be attached to the eyepiece, or a special eye-glass attached with elastics. In the latter any light filter can be used.

If the colorimeter is to be used as a spectro-photometer, a monochromatic eyepiece is substituted for the magnifying lense. It is also possible to connect the colorimeter to other spectro-photometers which can be used for monochromatic measurements.

**19. Kleinmann Micro-colorimeter.**<sup>13</sup>—In biological chemical work, the estimation of small amounts of substance is sometimes necessary, especially where it is necessary to work with small volumes. Kleinmann's micro-colorimeter is designed to estimate substances in 1 cc. of solution. The objection to most micro-colorimeters is that they use small depths of solution, thereby decreasing the accuracy. This objection is eliminated in Kleinmann's instrument by using very narrow and tall cups and plungers, approximately of 3.5 mm. diameter and 70 mm. depth.

*Description of Instrument.*—The instrument is built on the Duboscq principle, the optical and mechanical arrangement of both sides being perfectly symmetrical, as shown in Fig. 31.

(a) *Optical Construction.*—The light source may be an electrical bulb or illuminated ball, the latter being recommended. The light strikes a milk-glass plate,  $l$ , which diffuses the light on to two reflecting prisms,  $P_1$  and  $P_2$ . Figures with subtitle or index 1 refer to parts and light beam on one side of the instrument and 2 for the opposite side. The light from prism  $P$  passes through the solution  $f$  in cup  $F$  through plunger  $T$  to a reflection prism,  $p$ , then to a comparatory prism,  $v$ , through a blender,  $G$ , and then through the ocular or eyepiece including diaphragm  $A$  and lenses  $L_1$ ,  $L_2$ ,  $L_3$ , and  $L_4$ . • The terrestrial eyepiece with lenses and also the smaller aperture at  $O$  serves the purpose of increasing the length of the eyepiece and an enlargement of the field of vision.  $A$  and  $h_1$  and  $A$  and  $h_2$  are equidistant focuses, so that the observation of the light path by means of lens  $L_1$  and the light at  $T$ , a sharp picture of the opening of the diaphragm at  $A$  of  $h_1$  and  $h_2$  is obtained. The size of the opening at  $A$  is chosen so that the

<sup>13</sup> Biochem. Z. **179**, 276 (1926).

pictures of  $h_1$  and  $h_2$  are smaller than the diameter of the plunger  $T$  (3.5 mm.).

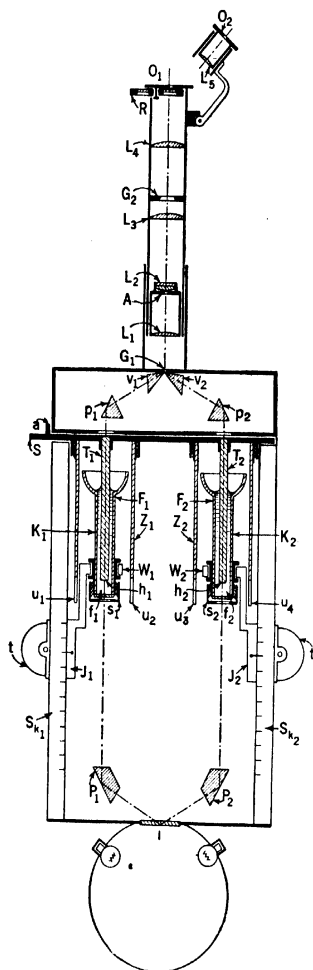


FIG. 31.

illumination without disturbing the observer with glares.

The base carries two racks,  $S_{K_1}$  and  $S_{K_2}$ , for moving the cups  $K_1$

The picture of the field at  $G_1$  is thrown by means of the ocular  $L_1-A$  on  $G_2$  and is seen by means of the terrestrial ocular  $L_4$  at the aperture  $O_1$ . At the same time a picture of the issuing light of the colorimeter is produced in the aperture which is smaller than the aperture  $O_2$ , thus throwing the same into the eye of the observer. In consequence  $h_1$  and  $h_2$  are pictured in the aperture  $O$ . With the adjustable lens  $L_5$  which is to be sharply focused, one can tell whether the surfaces  $h_1$  and  $h_2$  obstruct light by means of dirt or air bubbles. For this purpose the light from one side is cut off by a piece of dark paper or some other suitable opaque substance. By means of revolving disk  $R$ , color filters of yellow, red, green or blue may be inserted into the light path.

(b) *Mechanical Construction.*

—Figures 32 and 33 are pictures of the instrument. In front of the base of the instrument, the light ball of Schmidt and Haensch is easily attached with screws. The interior of the white ball carries two bulbs which require 4 volts (from storage cells). This produces a uniform, constant and bright

and  $K_2$  up and down. The racks carrying the pinions, to which is also attached the verniers of 0.1 mm., also have, on the side facing the observer, a millimeter scale. The movable platform contains metal rings or collars into which the cups  $K$  can be locked and from which they can also be easily removed. The cups contain 1 cc. when filled up to the mark and 3 cc. when filled to the top. The cups therefore have a working depth of between 60 and 70 mm. The metal clasp of the cups

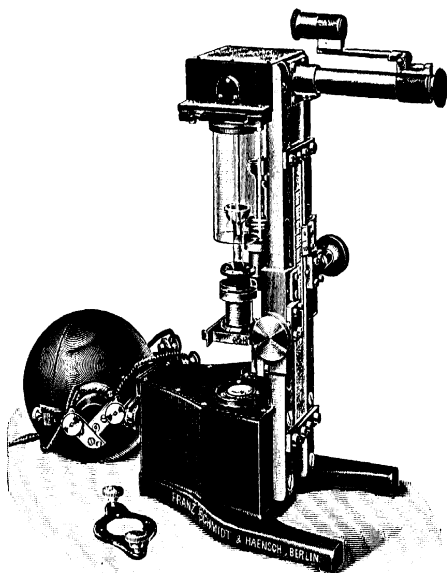


FIG. 32.—Kleinmann Micro-colorimeter.

contains a projection or plug which serves to orient the cups always in the same place. The exact placing of the cups is necessary as the light path on side 1 is displaced 1 mm. from that of 2 in order to secure a sharp dividing line in the halves of the field. The cups at their lower ends have rubber or leather washers to seal the bottom plates or disks.

The cups and their plungers,  $T$  are provided with glass protection cylinders,  $Z_1$  and  $Z_2$ , that are removable from the instrument by



unscrewing. The projection cylinders extend beyond the length of the plungers to the bottom of the cups when in zero position. These cylinders have at their lower ends at opposite sides two cut-outs which permit cleaning the plungers, while at the same time the protection cylinders prevent the breaking of the very fragile and expensive plungers. Another protection to the plungers to prevent their being damaged by the cups at zero position is a stop within 1 mm. of zero

position, which prevents the cups from touching the bottom of the plungers.

The plungers  $T_1$  and  $T_2$  and the protection cylinders  $Z_1$  and  $Z_2$  are arranged so that they are removed together by withdrawing the plate  $S$ . This permits easy cleaning of the plungers by using the protection cylinders as a rinsing flask. On reinsertion, a projection,  $a$ , insures that the correct position of the plungers is again obtained.

The errors of determination in using

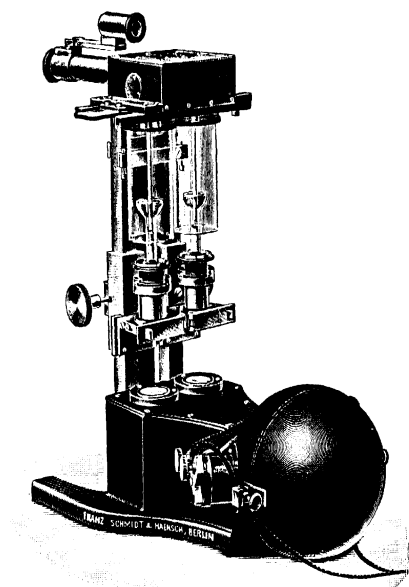


FIG. 33.—Kleinmann Micro-colorimeter.

this instrument, as given by Kleinmann, are, for solutions of about equal concentration, not more than 0.5 per cent for ten readings, and, for solutions of a concentration ratio of 1 : 2, for ten readings, not above 1.1 per cent.

**20. White Colorimeter.**<sup>14</sup>—Figure 34. The White Colorimeter consists essentially of two wedge-shaped hollow glass prisms of exactly equal dimensions and open at the large end for the introduction of the solutions to be tested. The wedges are held in a vertical posi-

<sup>14</sup> J. Am. Chem. Soc., **34**, 659 (1912).

tion side by side in a camera and may be raised or lowered by rack and pinion actuated by thumb screws. The prisms are screened from view on the side towards the operator except for a narrow horizontal slit across the middle of the camera through which the solutions are observed when a test is being made. The carriers are graduated to correspond to the length of the wedges, the zero of the scale being opposite the index when the sharp edge of the wedge is opposite the narrow opening in the screen through which the color is observed. The screens are adjustable so that the opening may be varied to suit the operator. The ground glass shutter at the forward end of the camera for diffusing the light is hinged in the manner of a door to facilitate the transfer of the wedges to and from the camera. The camera is mounted on a stand upon which it is free to turn in a horizontal plane, which renders it unnecessary to lift the instrument from its position while in use.

To carry out a determination with this instrument it is only necessary to dissolve and dilute to equal volumes equal quantities of the standard and of the material to be tested. Pour into the wedges convenient amounts of the two solutions, set the wedge containing the unknown at the graduation representing the percentage—or some multiple of it—of the coloring matter in the standard. Adjust the wedge containing the standard until the two agree in color. The percentage of coloring matter in the unknown is then indicated by the reading of the scale on the carrier containing the standard. Vertical sections through the two solutions parallel with the line of sight are similar triangles, the base of each being the thickness of solution at the point compared. It follows then that the readings on the graduated scales, since they represent the altitudes of these triangles, are measures which express the ratio existing between the amounts of coloring matter in the two solutions.

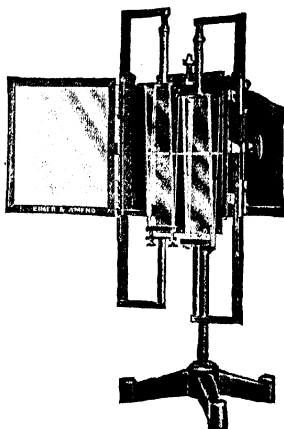


FIG. 34.—White Colorimeter.

The White Colorimeter has a number of good features: (1) It is so constructed that both eyes are used in making a test; (2) the eyes are protected by a camera from side lights; (3) the colored areas compared are close together; (4) uniform white light is visible around the colored spots compared; (5) the operator cannot see the graduated scales while making a comparison and, therefore, cannot be influenced by preconceived ideas; (6) an analysis may be checked by making readings at different points throughout the length of the wedges, especially in cases where the color is too deep or too pale for the most accurate comparison at the first position observed; (7) the wedges are easily emptied and filled so that passing from one determination to the next is quickly and easily effected; (8) the possibility of using any section of the wedge from its thinnest to its thickest part renders the apparatus adaptable to a wide range of determinations, and permits considerable variation in the amount of substance taken for an analysis; (9) the camera is mounted on a stand upon which it is free to turn in a horizontal plane, which makes it unnecessary to lift the instrument from its position while in use; and, finally, (10) the accuracy of the work done with the colorimeter appears to be limited only by the sensitivity of the eye to color changes. In the determination of carbon in steel, the maximum error obtained in a single reading was 0.6 per cent and this was considerably reduced by averaging several readings.<sup>15</sup>

While the wedge type colorimeter possesses the advantage of the individual wedge for a permanent standard, it has the disadvantages

that the individual wedges must be empirically standardized and that few of them are really permanent, and careful calibration of each wedge by the individual using the instrument is often omitted.

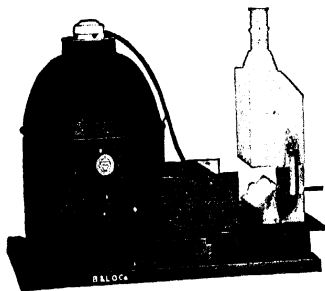


FIG. 35.—Improved Bausch & Lomb Colorimeter Lamp.

#### COLORIMETER LAMPS

**21. Improved Bausch & Lomb Colorimeter Lamp.**—Figure 35. This lamp produces a complete diffusion of reflected light over the entire surface of the colorimeter mirror.

<sup>15</sup> White, *loc. cit.*

Interchangeable blocks permit the use of instruments of different sizes.

The entire interior of the lamp is finished in dull white. This unpolished white surface of the wall provides illumination that is practically white, very evenly diffused and of uniform intensity. Within the dome-shaped top and out of direct line with the mirror of the colorimeters is a 60-watt Mazda bulb.

**22. New Palo Daylight Lamp. Cullen Model.**—Figure 35a. This lamp produces a true and natural North skylight and was designed especially for  $pH$  colorimetric work. The light from a special Mazda bulb is filtered through an accurate filter lens, the color composition of which has been carefully determined. The resultant North skylight rays are directed upon a reflector which increases the light diffusion and thus produces a uniform illumination.



**23. Cooper Hewitt Mercury Light.**—See Fig. 49, page 252.

**24. Schmidt & Haensch Sphere Illuminating Device.**—This illuminating device is designed for Schmidt & Haensch Colorimeters and consists of a hollow metal sphere, whitened inside and thus producing a diffuse reflecting surface which furnishes an absolutely uniform illumination. Two low-voltage bulbs mounted at proper angle inside the sphere serve as a source of light. Figure 30 shows the sphere illuminator attached to the colorimeter. This illuminator is also used with the Kleinmann micro-colorimeter. (See Figs. 32 and 33.)

FIG. 35a.—New Palo Daylight Lamp (Cullen Model).

# COLORIMETER TABLE

**25. Klett Result Table.**—This is a condensed table giving the result at 15 and 20 from 6 to 37 mm. It is printed on fine linen and is attached to rollers which can be moved forth and back very rapidly. A separate table printed on the same sheet is for the hemoglobin method in connection with the Newcomer hemoglobin standard. The table is shown on the base of the instrument in Fig. 18. It is also attached to the new Klett bio-colorimeter.

## CHAPTER III

### CALCULATION OF RESULTS <sup>1</sup>

**Standard Series Method.**—If a series of standards is used the amount of test substance in the sample is obtained directly by reading off the value of the standard which matches it in color. From this reading the percentage of test substance in the sample is easily calculated.

*Example:*

Weight of sample.....	2.0000 grams
Weight of test substance found.....	0.0010 gram

Therefore, sample contains  $\frac{0.0010}{2.0000} \times 100 = 0.05$  per cent test substance.

**Dilution Method.**—In this method the darker colored solution is diluted until the sample and standard solutions match in color when viewed *horizontally* through the tubes. At this point each solution contains the same *weight* of test substance per cubic centimeter. The amount of test substance in each is, therefore, directly proportional to their volumes.

*Example 1.*—Equal weights of sample and standard taken for the analysis.

Weight of sample.....	0.5000 gram
Weight of standard.....	0.5000 gram
Percentage of test substance in standard.....	0.25 gram
Reading of sample.....	50 cc.
Reading of standard.....	54 cc.

<sup>1</sup> All calculations in this chapter are based upon the assumption that Beer's law is obeyed. If the color reaction deviates from Beer's law, a correction curve should be constructed. A detailed discussion of colorimeter calibration and correction curves is given in Chapter IV.

Note that the *standard* solution was darker and hence it was diluted. Since equal weights of sample and standard were taken, the percentage of test substance in the sample may be calculated directly.

Thus we have,

$$\begin{array}{c} \text{Per cent} \\ \text{in sample} \end{array} : \begin{array}{c} \text{Per cent} \\ \text{in standard} \end{array} = \begin{array}{c} \text{Vol. of} \\ \text{sample} \end{array} : \begin{array}{c} \text{Vol. of} \\ \text{standard} \end{array}.$$

$$x : 0.25 = 50 : 54$$

$$x = \frac{0.25 \times 50}{54} = 0.23 \text{ per cent test substance in the sample.}$$

*Example 2.*—A solution containing a known weight of test substance per cubic centimeter is used as a standard.

Weight of sample..... 5.000 grams

Standard solution contains 0.00004 gram of test substance per cubic centimeter.

Reading of sample..... 32 cc.

Reading of standard..... 25 cc.

In this case the *sample* solution was darker and hence it was diluted.

Weight of test substance in standard is  $0.00004 \times 25 = 0.0010$  gram.

$$\begin{array}{c} \text{Weight of test} \\ \text{substance in sample} \end{array} : \begin{array}{c} \text{Weight of test} \\ \text{substance in standard} \end{array} = \begin{array}{c} \text{Vol. of} \\ \text{sample} \end{array} : \begin{array}{c} \text{Vol. of} \\ \text{standard} \end{array}.$$

$$x : 0.0010 = 32 : 25$$

$$x = \frac{0.0010 \times 32}{25} = 0.00128 \text{ gram of test substance contained}$$

in the sample.

$$\text{Therefore, } \frac{0.00128}{5.000} \times 100 = 0.026 \text{ per cent test substance in the}$$

sample.

**Duplication Method.**—In this method a relatively concentrated standard solution is measured into a “blank” containing the same reagents as used in the sample until the color is the same as that of the sample, after the volume of the standard has been brought up to the volume of the sample by the addition of distilled water. The volume

of standard solution required to prepare the "duplicate" is a measure of the amount of test substance in the sample.

*Example:*

Weight of sample..... 2.0000 grams

Standard required for duplication..... 4.6 cc.

Standard contained 0.0005 gram of test substance per cubic centimeter.

Total test substance required  $0.0005 \times 4.6 =$   
0.0023 gram.

Therefore,  $\frac{0.0023}{2.0000} \times 100 = 0.115$  per cent test substance contained

in the sample.

**Balancing Method.**—This method consists in placing the sample solution in a flat-bottom graduated tube and then running into another similar tube a standard color solution until the color intensities of the two are identical when viewed *vertically* through the length of the columns of liquids. When thus "balanced," the concentrations of the two solutions are *inversely* proportional to their *heights*.

*Example:*

Weight of sample..... 2.5000 grams

Volume of sample after solution..... 50 cc.

Standard solution contains 0.00002 gram of test substance per cubic centimeter.

Height of sample solution in tube *A* = 50 mm.

Height of standard solution in tube *B* = 58 mm.

Weight of test substance : Weight of test substance = Height of soln. in *B* : Height of soln. in *A*.  
per cc. in *A*. per cc. in *B*.

$$x : 0.00002 = 58 : 50$$

$$x = \frac{0.00002 \times 58}{50} = 0.0000232 \text{ gram of test substance per}$$

cubic centimeter of the sample solution.

Total test substance in sample is, therefore,  $0.0000232 \times 50 =$   
0.00115 gram.

Hence,  $\frac{0.00115}{2.5000} \times 100 = 0.046$  per cent test substance in the sample.

**Simplified Calculations in Colorimetry.**—In the above paragraphs we have illustrated the calculation of results obtained by each of the four common colorimetric methods, namely, standard series, dilution, duplication, and balancing. McCrackan, Passamaneck, and Harman<sup>2</sup> have recently called attention to a simplified method of calculation in colorimetry when the color matching is made with a colorimeter, whether the instrument be of the single plunger, the two plunger, or the dilution type. In each case a reading on the known ( $K$ ) and a reading on the unknown ( $U$ ) have to be taken.

“ These readings become the terms of fractions used in obtaining factors that form a part of calculation formulas,  $K/U$  being used for plunger type colorimeters, and  $U/K$  for those of the dilution type. These ratios are constant throughout a comparison in a colorimeter, and since they are used as factors they may be represented by  $F$  in the expressions:

$$K/U = F, \text{ and } U/K = F.$$

“ If any fixed value be assigned to  $K$ , then  $U$  will assume a corresponding value, and vice versa. As the analyst can choose which term he will make constant, and can choose any value within wide limits, it is evidently advantageous to choose a whole number, and one expressible by one significant figure, for the divisor, such as 8, 10, 20, or 30. By so doing he can make all division short. He should, therefore, set the unknown in applying a plunger type colorimeter, and get a reading for the known, and in applying the dilution type colorimeter, vice versa. In using 15 as a divisor, as one would be compelled to do in using a 15 mm. Bock-Benedict cell for the unknown, he could divide successively by 3 and 5, the factors of 15, and avoid long division, and a similar procedure could be followed with any other factorable number.

“ Most writers on colorimetry assume the use of the plunger type colorimeter for exact work, and they usually direct that the known be given a value, and that a reading for the unknown be made after the fields are matched. The number 20 is most often used for the known, and the readings for the unknown, made to one decimal place usually, give values that make long division necessary, and the factors obtained by the division usually have to be rounded off to prevent the use of figures to more than the third decimal place.

<sup>2</sup> J. Chem. Ed., **3**, 416 (1926).



TABLE I  
(McCrackan, Passamaneck and Harman)

All values for  $X/20$  are shown when  $X$  varies from 10.0 to 45.9.

In both the calculation formulas, Known/Unknown =  $P$ , and Unknown/Known =  $F$ , the dividend is represented by  $X$ , and the divisor by 20.

$X$  is the dividend in  $K/U$ , and in  $U/K$

$X$	$X/20$	$X$	$X/20$	$X$	$X/20$	$X$	$X/20$	$X$	$X/20$	$X$	$X/20$	$X$	$X/20$
10.0	0.500	16.0	0.800	22.0	1.100	28.0	1.400	34.0	1.700	40.0	2.000		
10.1	0.505	16.1	0.805	22.1	1.105	28.1	1.405	34.1	1.705	40.1	2.005		
10.2	0.510	16.2	0.810	22.2	1.110	28.2	1.410	34.2	1.710	40.2	2.010		
10.3	0.515	16.3	0.815	22.3	1.115	28.3	1.415	34.3	1.715	40.3	2.015		
10.4	0.520	16.4	0.820	22.4	1.120	28.4	1.420	34.4	1.720	40.4	2.020		
10.5	0.525	16.5	0.825	22.5	1.125	28.5	1.425	34.5	1.725	40.5	2.025		
10.6	0.530	16.6	0.830	22.6	1.130	28.6	1.430	34.6	1.730	40.6	2.030		
10.7	0.535	16.7	0.835	22.7	1.135	28.7	1.435	34.7	1.735	40.7	2.035		
10.8	0.540	16.8	0.840	22.8	1.140	28.8	1.440	34.8	1.740	40.8	2.040		
10.9	0.545	16.9	0.845	22.9	1.145	28.9	1.445	34.9	1.745	40.9	2.045		
11.0	0.550	17.0	0.850	23.0	1.150	29.0	1.450	35.0	1.750	41.0	2.050		
11.1	0.555	17.1	0.855	23.1	1.155	29.1	1.455	35.1	1.755	41.1	2.055		
11.2	0.560	17.2	0.860	23.2	1.160	29.2	1.460	35.2	1.760	41.2	2.060		
11.3	0.565	17.3	0.865	23.3	1.165	29.3	1.465	35.3	1.765	41.3	2.065		
11.4	0.570	17.4	0.870	23.4	1.170	29.4	1.470	35.4	1.770	41.4	2.070		
11.5	0.575	17.5	0.875	23.5	1.175	29.5	1.475	35.5	1.775	41.5	2.075		
11.6	0.580	17.6	0.880	23.6	1.180	29.6	1.480	35.6	1.780	41.6	2.080		
11.7	0.585	17.7	0.885	23.7	1.185	29.7	1.485	35.7	1.785	41.7	2.085		
11.8	0.590	17.8	0.890	23.8	1.190	29.8	1.490	35.8	1.790	41.8	2.090		
11.9	0.595	17.9	0.895	23.9	1.195	29.9	1.495	35.9	1.795	41.9	2.095		
12.0	0.600	18.0	0.900	24.0	1.200	30.0	1.500	36.0	1.800	42.0	2.100		
12.1	0.605	18.1	0.905	24.1	1.205	30.1	1.505	36.1	1.805	42.1	2.105		
12.2	0.610	18.2	0.910	24.2	1.210	30.2	1.510	36.2	1.810	42.2	2.110		
12.3	0.615	18.3	0.915	24.3	1.215	30.3	1.515	36.3	1.815	42.3	2.115		
12.4	0.620	18.4	0.920	24.4	1.220	30.4	1.520	36.4	1.820	42.4	2.120		
12.5	0.625	18.5	0.925	24.5	1.225	30.5	1.525	36.5	1.825	42.5	2.125		
12.6	0.630	18.6	0.930	24.6	1.230	30.6	1.530	36.6	1.830	42.6	2.130		
12.7	0.635	18.7	0.935	24.7	1.235	30.7	1.535	36.7	1.835	42.7	2.135		
12.8	0.640	18.8	0.940	24.8	1.240	30.8	1.540	36.8	1.840	42.8	2.140		
12.9	0.645	18.9	0.945	24.9	1.245	30.9	1.545	36.9	1.845	42.9	2.145		
13.0	0.650	19.0	0.950	25.0	1.250	31.0	1.550	37.0	1.850	43.0	2.150		
13.1	0.655	19.1	0.955	25.1	1.255	31.1	1.555	37.1	1.855	43.1	2.155		
13.2	0.660	19.2	0.960	25.2	1.260	31.2	1.560	37.2	1.860	43.2	2.160		
13.3	0.665	19.3	0.965	25.3	1.265	31.3	1.565	37.3	1.865	43.3	2.165		
13.4	0.670	19.4	0.970	25.4	1.270	31.4	1.570	37.4	1.870	43.4	2.170		
13.5	0.675	19.5	0.975	25.5	1.275	31.5	1.575	37.5	1.875	43.5	2.175		
13.6	0.680	19.6	0.980	25.6	1.280	31.6	1.580	37.6	1.880	43.6	2.180		
13.7	0.685	19.7	0.985	25.7	1.285	31.7	1.585	37.7	1.885	43.7	2.185		
13.8	0.690	19.8	0.990	25.8	1.290	31.8	1.590	37.8	1.890	43.8	2.190		
13.9	0.695	19.9	0.995	25.9	1.295	31.9	1.595	37.9	1.895	43.9	2.195		
14.0	0.700	20.0	1.000	26.0	1.300	32.0	1.600	38.0	1.900	44.0	2.200		
14.1	0.705	20.1	1.005	26.1	1.305	32.1	1.605	38.1	1.905	44.1	2.205		
14.2	0.710	20.2	1.010	26.2	1.310	32.2	1.610	38.2	1.910	44.2	2.210		
14.3	0.715	20.3	1.015	26.3	1.315	32.3	1.615	38.3	1.915	44.3	2.215		
14.4	0.720	20.4	1.020	26.4	1.320	32.4	1.620	38.4	1.920	44.4	2.220		
14.5	0.725	20.5	1.025	26.5	1.325	32.5	1.625	38.5	1.925	44.5	2.225		
14.6	0.730	20.6	1.030	26.6	1.330	32.6	1.630	38.6	1.930	44.6	2.230		
14.7	0.735	20.7	1.035	26.7	1.335	32.7	1.635	38.7	1.935	44.7	2.235		
14.8	0.740	20.8	1.040	26.8	1.340	32.8	1.640	38.8	1.940	44.8	2.240		
14.9	0.745	20.9	1.045	26.9	1.345	32.9	1.645	38.9	1.945	44.9	2.245		
15.0	0.750	21.0	1.050	27.0	1.350	33.0	1.650	39.0	1.950	45.0	2.250		
15.1	0.755	21.1	1.055	27.1	1.355	33.1	1.655	39.1	1.955	45.1	2.255		
15.2	0.760	21.2	1.060	27.2	1.360	33.2	1.660	39.2	1.960	45.2	2.260		
15.3	0.765	21.3	1.065	27.3	1.365	33.3	1.665	39.3	1.965	45.3	2.265		
15.4	0.770	21.4	1.070	27.4	1.370	33.4	1.670	39.4	1.970	45.4	2.270		
15.5	0.775	21.5	1.075	27.5	1.375	33.5	1.675	39.5	1.975	45.5	2.275		
15.6	0.780	21.6	1.080	27.6	1.380	33.6	1.680	39.6	1.980	45.6	2.280		
15.7	0.785	21.7	1.085	27.7	1.385	33.7	1.685	39.7	1.985	45.7	2.285		
15.8	0.790	21.8	1.090	27.8	1.390	33.8	1.690	39.8	1.990	45.8	2.290		
15.9	0.795	21.9	1.095	27.9	1.395	33.9	1.695	39.9	1.995	45.9	2.295		

"The Klett Mfg. Co. has copyrighted tables prepared by Benedict showing values rounded off to three decimal places for values of  $K/U$  when  $K$  is 15, and  $U$  is 6.0 to 29.9, and when  $K$  is 20, and  $U$  is 8.0 to 32.9. Mathews<sup>3</sup> includes tables from Hulton-Frankel in his latest text, showing creatinine and sugar percentages in blood for readings for the unknowns when the known is set at 20, and Falisi and Lawton<sup>4</sup> give tables for calculating creatinine, non-protein nitrogen, urea nitrogen, sugar, and uric acid for readings for the unknowns when the known is set at 20. All such tables could more easily be prepared for readings on the knowns when the unknowns are set, and the ease with which calculations could be verified would make work less empirical."

In Table I are shown all values for  $K/U$  and  $U/K$  when the divisor is made 20 and the dividend varies from 10.0 to 45.9. None of these factors are rounded off, yet only 50 per cent of them require as many as three decimal places. They are absolutely exact, and all are obtained by short division, making it possible for students and analysts to verify calculations with little loss of time and energy.

Where most accurate work is done with colorimeters reading to the second decimal place, the wisdom of having a divisor with one significant figure is still more apparent. With such instruments and the unknown set on 20, factors absolutely accurate to the fourth decimal place, from an arithmetical point of view, can be obtained, and only 50 per cent of them need four decimals to express them. A table giving these factors would be just ten times as long as the preceding three-place table based on readings made to one place.

#### TABLES OF GENERAL APPLICATION FOR VERIFYING COMPLETE COLORIMETRIC CALCULATIONS<sup>5</sup>

It is well known that formulas for colorimetric calculations can be divided into two factors, one of which is constant for any fixed analytic procedure, and the other of which varies with the colorimetric readings.

<sup>3</sup> A. P. Mathews, *Physiological Chemistry*, 4th ed., pp. 1073-74. William Wood & Co., New York, 1925.

<sup>4</sup> J. C. Falisi and Vera L. Lawton, *Tables for Blood Chemistry Calculations*, *J. Lab. Clin. Med.*, **9**, 566 (1924).

<sup>5</sup> R. F. McCrackan, Kate E. Harman, and E. Passamaneck, *Arch. Path. Lab. Med.*, **3**, 227 (1927).

It has been suggested that  $C^0$  represent the constant factor, and that the variable factor be represented by  $F$  (see p. 55) whether it represents the reading for the known divided by that for the unknown when a colorimeter of the plunger type is used, or the reading for the unknown divided by that for the known when the colorimeter is of the dilution type. Then when  $W$  represents the weight of the unknown substance sought,

$$FC = W$$

It has been shown that the division necessary to obtain  $F$  can be made short, and that repeating decimals can be avoided, by choosing such values as 20, 10 and 25 for the divisors. The purpose of the following paragraphs is to explain the use of tables showing values for  $F$ , and for  $W$  or  $FC$ , for all readings to one decimal place between 13.0 and 30.9 when the divisor is 20; between 16.0 and 37.9 when the divisor is 25, and between 6.0 and 15.9 when the divisor is 10. The tables are so much alike in principle that only one of them (Table III) will be explained.

Table III, which will be most used, probably, is for use only when the unknown is set on 20 for a colorimeter of the plunger type, or the known is 20 in volume for one of the dilution type. The numbers in the first column, marked  $D$ , which means dividend, are the integral parts of the colorimeter readings for the dividends that are to be divided by 20, and the fractional parts of the dividends are across the top of the table. In the second column, marked  $C$ , meaning con-

<sup>6</sup> This constant factor  $C$  is equal to  $SVV'/PV''$  in the equation:

$$KSNV'/UPV'' = W$$

where  $P$  = the portion of solution taken for analysis;

$S$  = the weight of standard substance in the known solution;

$V'$  = the volume of the unknown solution;

$V''$  = the volume of the known solution;

$U$  = the colorimeter reading for the unknown solution;

$K$  = the colorimeter reading for the known solution;

$N$  = the number used as a factor to express the analytical results on a conventional basis, and

$W$  = the weight of the standard substance in the unknown solution expressed on the conventional basis indicated in the choice of the number  $N$ , above.

$P$ ,  $V'$ , and  $V''$  are usually expressed in cubic centimeters,  $S$  and  $W$  in milligrams,  $U$  and  $K$  in colorimeter units, and  $N$  as an abstract number.

For a study of the mathematics of colorimetry by means of the above general formula, see McCrackan, J. Chem. Ed., **3**, 928 (1926).

stant factors, figures from 1 to 9 are repeated with each whole number found in the first column. Under the fractional parts of the dividends, and opposite 1 of the second column, in each case, the value of  $F$ , or of dividend divided by divisor, is found, and under these, opposite the other numbers of the second column are the products of  $F$  and  $C$ , or  $W$ . All values are shown for  $FC$  or  $W$ , when  $C$  is a whole number between 1 and 9. In case  $C$  is a decimal fraction between 0.001 and 0.900 with but one significant figure, or a number above 9 with but one significant figure, the figures of the tables still show the values sought, provided that the decimal point is moved correctly in each case. In most procedures the value of  $C$  contains only one significant figure, but when it contains two figures  $W$  can still be found by means of the table by pointing off two numbers correctly, and adding them.

While the table can be used in calculations for any fixed colorimetric procedures, Folin's<sup>7</sup> well-known methods for analyzing protein-free blood filtrates will be taken to illustrate its use. Let the colorimeter be of the plunger type, the weight of glucose used as the standard be 0.4 mg., and the reading for the known be 19.7, when the unknown is set on 20, to find the number of milligrams of glucose in 100 cc. of blood. The general formula applicable is:

$$\frac{\text{Reading on Known}}{\text{Reading on Unknown}} \times \frac{\text{Vol. Unknown}}{\text{Vol. Known}} \times \frac{\text{Mg. of Standard}}{\text{Vol. Blood Filtrate Analyzed}} \times 1000 = W$$

$$\frac{19.7}{20} \times \frac{25}{25} \times \frac{0.4}{2} \times 1000 = 197$$

A glance at the left member of the equation shows that 19.7/20 is  $F$ , the variable factor. The balance of the left member of the equation is the constant factor  $C$  for the analytic procedure, and it equals 200. The significant figure in 200 is 2, and opposite 2 of the second column, under 19.7, the table shows 1.970. To get the correct value for 200, the decimal point must be moved two places to the right. This gives 197, checking the value found by calculation. The value of  $F$ , or

<sup>7</sup> O. Folin, *Laboratory Manual of Biological Chemistry*, pp. 227-277. D. Appleton & Co., New York, 1926; O. Folin and H. Wu, *A System of Blood Analysis*, *J. Biol. Chem.*, **38**, 81 (1919).

19.7/20, which is found to be 0.985 by calculation, can be verified by looking under 19.7, opposite 1 of the second column.

The values for  $C$  in all of Folin's procedures have been calculated and tabulated for use when needed. For his weaker standards they are 4, 30, 15 and 1.5 for uric acid, nonprotein nitrogen, urea nitrogen, and creatinine, respectively. Let the unknowns be set on 20, and let the readings for the knowns in these four determinations be 19.4, 19.6, 20.3 and 20.5, respectively, to find the weights of these substances in 100 cc. of blood. For uric acid, under 19.4, opposite 4 of the second column, the answer 3.88 is found. For nonprotein nitrogen, under 19.6, opposite 3, the figures 2.94 become 29.4, the answer, by moving the point one place. In finding the urea nitrogen, 15, which contains two significant figures, must be used for  $C$ . The one represents ten, and the five represents units, so opposite 1, under 20.3, the number 1.015 is changed to 10.15 and added to 5.075 found just below, opposite 5 of the second column, giving 15.225 as the unabbreviated answer. Similarly with the creatinine, where  $C$  is 1.5, the number under 20.5, opposite 1, is 1.025; the number opposite 5, divided by 10, is 0.5125, and the sum of the two numbers is 1.5375, which might be recorded as 1.54 mg.

While calculations, made in the old ways, can be done away with, and these tables used instead, this is not recommended, and particularly not in the work of technicians and students. Calculations should be made independently, and the tables used as a means of checking the arithmetic. Moreover, it must be understood that these tables are based upon the assumption of the validity of Beer's law, at least over the range in concentration employed, and that for all methods which deviate from this law, correction curves or factors must be obtained in the usual way. (See Chapter IV.)

TABLE II  
(McCracken, Harman and Passamaneck)VALUES FOR DIVIDENDS DIVIDED BY 10 AND MULTIPLIED BY FROM 1 TO 9  
WHEN COLORIMETER READINGS RANGE FROM 6.0 TO 15.9

D	C	0.6	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
6	1	0.60	0.61	0.62	0.63	0.64	0.65	0.66	0.67	0.68	0.69
	2	1.20	1.22	1.24	1.26	1.28	1.30	1.32	1.34	1.36	1.38
	3	1.80	1.83	1.86	1.89	1.92	1.95	1.98	2.01	2.04	2.07
	4	2.40	2.44	2.48	2.52	2.56	2.60	2.64	2.68	2.72	2.76
	5	3.00	3.05	3.10	3.15	3.20	3.25	3.30	3.35	3.40	3.45
	6	3.60	3.66	3.72	3.78	3.84	3.90	3.96	4.02	4.08	4.14
	7	4.20	4.27	4.34	4.41	4.48	4.55	4.62	4.69	4.76	4.83
	8	4.80	4.88	4.96	5.04	5.12	5.20	5.28	5.36	5.44	5.52
	9	5.40	5.49	5.58	5.67	5.76	5.85	5.94	6.03	6.12	6.21
7	1	0.70	0.71	0.72	0.73	0.74	0.75	0.76	0.77	0.78	0.79
	2	1.40	1.42	1.44	1.46	1.48	1.50	1.52	1.54	1.56	1.58
	3	2.10	2.13	2.16	2.19	2.22	2.25	2.28	2.31	2.34	2.37
	4	2.80	2.84	2.88	2.92	2.96	3.00	3.04	3.08	3.12	3.16
	5	3.50	3.55	3.60	3.65	3.70	3.75	3.80	3.85	3.90	3.95
	6	4.20	4.26	4.32	4.38	4.44	4.50	4.56	4.62	4.68	4.74
	7	4.90	4.97	5.04	5.11	5.18	5.25	5.32	5.39	5.46	5.53
	8	5.60	5.68	5.76	5.84	5.92	6.00	6.08	6.16	6.24	6.32
	9	6.30	6.39	6.48	6.57	6.66	6.75	6.84	6.93	7.02	7.11
8	1	0.80	0.81	0.82	0.83	0.84	0.85	0.86	0.87	0.88	0.89
	2	1.60	1.62	1.64	1.66	1.68	1.70	1.72	1.74	1.76	1.78
	3	2.40	2.43	2.46	2.49	2.52	2.55	2.58	2.61	2.64	2.67
	4	3.20	3.24	3.28	3.32	3.36	3.40	3.44	3.48	3.52	3.56
	5	4.00	4.05	4.10	4.15	4.20	4.25	4.30	4.35	4.40	4.45
	6	4.80	4.86	4.92	4.98	5.04	5.10	5.16	5.22	5.28	5.34
	7	5.60	5.67	5.74	5.81	5.88	5.95	6.02	6.09	6.16	6.23
	8	6.40	6.48	6.56	6.64	6.72	6.80	6.88	6.96	7.04	7.12
	9	7.20	7.29	7.38	7.47	7.56	7.65	7.74	7.83	7.92	8.01
9	1	0.90	0.91	0.92	0.93	0.94	0.95	0.96	0.97	0.98	0.99
	2	1.80	1.82	1.84	1.86	1.88	1.90	1.92	1.94	1.96	1.98
	3	2.70	2.73	2.76	2.79	2.82	2.85	2.88	2.91	2.94	2.97
	4	3.60	3.64	3.68	3.72	3.76	3.80	3.84	3.88	3.92	3.96
	5	4.50	4.55	4.60	4.65	4.70	4.75	4.80	4.85	4.90	4.95
	6	5.40	5.46	5.52	5.58	5.64	5.70	5.76	5.82	5.88	5.94
	7	6.30	6.37	6.44	6.51	6.58	6.65	6.72	6.79	6.86	6.93
	8	7.20	7.28	7.36	7.44	7.52	7.60	7.68	7.76	7.84	7.92
	9	8.10	8.19	8.28	8.37	8.46	8.55	8.64	8.73	8.82	8.91
10	1	1.00	1.01	1.02	1.03	1.04	1.05	1.06	1.07	1.08	1.09
	2	2.00	2.02	2.04	2.06	2.08	2.10	2.12	2.14	2.16	2.18
	3	3.00	3.03	3.06	3.09	3.12	3.15	3.18	3.21	3.24	3.27
	4	4.00	4.04	4.08	4.12	4.16	4.20	4.24	4.28	4.32	4.36
	5	5.00	5.05	5.10	5.15	5.20	5.25	5.30	5.35	5.40	5.45
	6	6.00	6.06	6.12	6.18	6.24	6.30	6.36	6.42	6.48	6.54
	7	7.00	7.07	7.14	7.21	7.28	7.35	7.42	7.49	7.56	7.63
	8	8.00	8.08	8.16	8.24	8.32	8.40	8.48	8.56	8.64	8.72
	9	9.00	9.09	9.18	9.27	9.36	9.45	9.54	9.63	9.72	9.81

TABLE II—Continued

<i>D</i>	<i>C</i>	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
11	1	1.10	1.11	1.12	1.13	1.14	1.15	1.16	1.17	1.18	1.19
	2	2.20	2.22	2.24	2.26	2.28	2.30	2.32	2.34	2.36	2.38
	3	3.30	3.33	3.36	3.39	3.42	3.45	3.48	3.51	3.54	3.57
	4	4.40	4.44	4.48	4.52	4.56	4.60	4.64	4.68	4.72	4.76
	5	5.50	5.55	5.60	5.65	5.70	5.75	5.80	5.85	5.90	5.95
	6	6.60	6.66	6.72	6.78	6.84	6.90	6.96	7.02	7.08	7.14
	7	7.70	7.77	7.84	7.91	7.98	8.05	8.12	8.19	8.26	8.33
	8	8.80	8.88	8.96	9.04	9.12	9.20	9.28	9.36	9.44	9.52
	9	9.90	9.99	10.08	10.17	10.26	10.35	10.44	10.53	10.62	10.71
12	1	1.20	1.21	1.22	1.23	1.24	1.25	1.26	1.27	1.28	1.29
	2	2.40	2.42	2.44	2.46	2.48	2.50	2.52	2.54	2.56	2.58
	3	3.60	3.63	3.66	3.69	3.72	3.75	3.78	3.81	3.84	3.87
	4	4.80	4.84	4.88	4.92	4.96	5.00	5.04	5.08	5.12	5.16
	5	6.00	6.05	6.10	6.15	6.20	6.25	6.30	6.35	6.40	6.45
	6	7.20	7.26	7.32	7.38	7.44	7.50	7.56	7.62	7.68	7.74
	7	8.40	8.47	8.54	8.61	8.68	8.75	8.82	8.89	8.96	9.03
	8	9.60	9.68	9.76	9.84	9.92	10.00	10.08	10.16	10.24	10.32
	9	10.80	10.89	10.98	11.07	11.16	11.25	11.34	11.43	11.52	11.61
13	1	1.30	1.31	1.32	1.33	1.34	1.35	1.36	1.37	1.38	1.39
	2	2.60	2.62	2.64	2.66	2.68	2.70	2.72	2.74	2.76	2.78
	3	3.90	3.93	3.96	3.99	4.02	4.05	4.08	4.11	4.14	4.17
	4	5.20	5.24	5.28	5.32	5.36	5.40	5.44	5.48	5.52	5.56
	5	6.50	6.55	6.60	6.65	6.70	6.75	6.80	6.85	6.90	6.95
	6	7.80	7.86	7.92	7.98	8.04	8.10	8.16	8.22	8.28	8.34
	7	9.10	9.17	9.24	9.31	9.38	9.45	9.52	9.59	9.66	9.73
	8	10.40	10.48	10.56	10.64	10.72	10.80	10.88	10.96	11.04	11.12
	9	11.70	11.79	11.88	11.97	12.06	12.15	12.24	12.33	12.42	12.51
14	1	1.40	1.41	1.42	1.43	1.44	1.45	1.46	1.47	1.48	1.49
	2	2.80	2.82	2.84	2.86	2.88	2.90	2.92	2.94	2.96	2.98
	3	4.20	4.23	4.26	4.29	4.32	4.35	4.38	4.41	4.44	4.47
	4	5.60	5.64	5.68	5.72	5.76	5.80	5.84	5.88	5.92	5.96
	5	7.00	7.05	7.10	7.15	7.20	7.25	7.30	7.35	7.40	7.45
	6	8.40	8.46	8.52	8.58	8.64	8.70	8.76	8.82	8.88	8.94
	7	9.80	9.87	9.94	10.01	10.08	10.15	10.22	10.29	10.36	10.43
	8	11.20	11.28	11.36	11.44	11.52	11.60	11.68	11.76	11.84	11.92
	9	12.60	12.69	12.78	12.87	12.96	13.05	13.14	13.23	13.32	13.41
15	1	1.50	1.51	1.52	1.53	1.54	1.55	1.56	1.57	1.58	1.59
	2	3.00	3.02	3.04	3.06	3.08	3.10	3.12	3.14	3.16	3.18
	3	4.50	4.53	4.56	4.59	4.62	4.65	4.68	4.71	4.74	4.77
	4	6.00	6.04	6.08	6.12	6.16	6.20	6.24	6.28	6.32	6.36
	5	7.50	7.55	7.60	7.65	7.70	7.75	7.80	7.85	7.90	7.95
	6	9.00	9.06	9.12	9.18	9.24	9.30	9.36	9.42	9.48	9.54
	7	10.50	10.57	10.64	10.71	10.78	10.85	10.92	10.99	11.06	11.13
	8	12.00	12.08	12.16	12.24	12.32	12.40	12.48	12.56	12.64	12.72
	9	13.50	13.59	13.68	13.77	13.86	13.95	14.04	14.13	14.22	14.31

TABLE III

VALUES FOR DIVIDENDS DIVIDED BY 20 AND MULTIPLIED BY 1 TO 9

WHEN COLORIMETER READINGS RANGE FROM 13.0 TO 30.9 \*

D	C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
13	1	0.650	0.655	0.660	0.665	0.670	0.675	0.680	0.685	0.690	0.695
	2	1.300	1.310	1.320	1.330	1.340	1.350	1.360	1.370	1.380	1.390
	3	1.950	1.965	1.980	1.995	2.010	2.025	2.040	2.055	2.070	2.085
	4	2.600	2.620	2.640	2.660	2.680	2.700	2.720	2.740	2.760	2.780
	5	3.250	3.275	3.300	3.325	3.350	3.375	3.400	3.425	3.450	3.475
	6	3.900	3.930	3.960	3.990	4.020	4.050	4.080	4.110	4.140	4.170
	7	4.550	4.585	4.620	4.655	4.690	4.725	4.760	4.795	4.830	4.865
	8	5.200	5.240	5.280	5.320	5.360	5.400	5.440	5.480	5.520	5.560
	9	5.850	5.895	5.940	5.985	6.030	6.075	6.120	6.165	6.210	6.255
14	1	0.700	0.705	0.710	0.715	0.720	0.725	0.730	0.735	0.740	0.745
	2	1.400	1.410	1.420	1.430	1.440	1.450	1.460	1.470	1.480	1.490
	3	2.100	2.115	2.130	2.145	2.160	2.175	2.190	2.205	2.220	2.235
	4	2.800	2.820	2.840	2.860	2.880	2.900	2.920	2.940	2.960	2.980
	5	3.500	3.525	3.550	3.575	3.600	3.625	3.650	3.675	3.700	3.725
	6	4.200	4.230	4.260	4.290	4.320	4.350	4.380	4.410	4.440	4.470
	7	4.900	4.935	4.970	5.005	5.040	5.075	5.110	5.145	5.180	5.215
	8	5.600	5.640	5.680	5.720	5.760	5.800	5.840	5.880	5.920	5.960
	9	6.300	6.345	6.390	6.435	6.480	6.525	6.570	6.615	6.660	6.705
15	1	0.750	0.755	0.760	0.765	0.770	0.775	0.780	0.785	0.790	0.795
	2	1.500	1.510	1.520	1.530	1.540	1.550	1.560	1.570	1.580	1.590
	3	2.250	2.265	2.280	2.295	2.310	2.325	2.340	2.355	2.370	2.385
	4	3.000	3.020	3.040	3.060	3.080	3.100	3.120	3.140	3.160	3.180
	5	3.750	3.775	3.800	3.825	3.850	3.875	3.900	3.925	3.950	3.975
	6	4.500	4.530	4.560	4.590	4.620	4.650	4.680	4.710	4.740	4.770
	7	5.250	5.285	5.320	5.355	5.390	5.425	5.460	5.495	5.530	5.565
	8	6.000	6.040	6.080	6.120	6.160	6.200	6.240	6.280	6.320	6.360
	9	6.750	6.795	6.840	6.885	6.930	6.975	7.020	7.065	7.110	7.155
16	1	0.800	0.805	0.810	0.815	0.820	0.825	0.830	0.835	0.840	0.845
	2	1.600	1.610	1.620	1.630	1.640	1.650	1.660	1.670	1.680	1.690
	3	2.400	2.415	2.430	2.445	2.460	2.475	2.490	2.505	2.520	2.535
	4	3.200	3.220	3.240	3.260	3.280	3.300	3.320	3.340	3.360	3.380
	5	4.000	4.025	4.050	4.075	4.100	4.125	4.150	4.175	4.200	4.225
	6	4.800	4.830	4.860	4.890	4.920	4.950	4.980	5.010	5.040	5.070
	7	5.600	5.635	5.670	5.705	5.740	5.775	5.810	5.845	5.880	5.915
	8	6.400	6.440	6.480	6.520	6.560	6.600	6.640	6.680	6.720	6.760
	9	7.200	7.245	7.290	7.335	7.380	7.425	7.470	7.515	7.560	7.605
17	1	0.850	0.855	0.860	0.865	0.870	0.875	0.880	0.885	0.890	0.895
	2	1.700	1.710	1.720	1.730	1.740	1.750	1.760	1.770	1.780	1.790
	3	2.550	2.565	2.580	2.595	2.610	2.625	2.640	2.655	2.670	2.685
	4	3.400	3.420	3.440	3.460	3.480	3.500	3.520	3.540	3.560	3.580
	5	4.250	4.275	4.300	4.325	4.350	4.375	4.400	4.425	4.450	4.475
	6	5.100	5.130	5.160	5.190	5.220	5.250	5.280	5.310	5.340	5.370
	7	5.950	5.985	6.020	6.055	6.090	6.125	6.160	6.195	6.230	6.265
	8	6.800	6.840	6.880	6.920	6.960	7.000	7.040	7.080	7.120	7.160
	9	7.650	7.695	7.740	7.785	7.830	7.875	7.920	7.965	8.010	8.055
18	1	0.900	0.905	0.910	0.915	0.920	0.925	0.930	0.935	0.940	0.945
	2	1.800	1.810	1.820	1.830	1.840	1.850	1.860	1.870	1.880	1.890
	3	2.700	2.715	2.730	2.745	2.760	2.775	2.790	2.805	2.820	2.835
	4	3.600	3.620	3.640	3.660	3.680	3.700	3.720	3.740	3.760	3.780
	5	4.500	4.525	4.550	4.575	4.600	4.625	4.650	4.675	4.700	4.725
	6	5.400	5.430	5.460	5.490	5.520	5.550	5.580	5.610	5.640	5.670
	7	6.300	6.335	6.370	6.405	6.440	6.475	6.510	6.545	6.580	6.615
	8	7.200	7.240	7.280	7.320	7.360	7.400	7.440	7.480	7.520	7.560
	9	8.100	8.145	8.190	8.235	8.280	8.325	8.370	8.415	8.460	8.505



TABLE III—Continued

<i>D</i>	<i>C</i>	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
19	1	0.950	0.955	0.960	0.965	0.970	0.975	0.980	0.985	0.990	0.995
	2	1.900	1.910	1.920	1.930	1.940	1.950	1.960	1.970	1.980	1.990
	3	2.850	2.865	2.880	2.895	2.910	2.925	2.940	2.955	2.970	2.985
	4	3.800	3.820	3.840	3.860	3.880	3.900	3.920	3.940	3.960	3.980
	5	4.750	4.775	4.800	4.825	4.850	4.875	4.900	4.925	4.950	4.975
	6	5.700	5.730	5.760	5.790	5.820	5.850	5.880	5.910	5.940	5.970
	7	6.650	6.685	6.720	6.755	6.790	6.825	6.860	6.895	6.930	6.965
	8	7.600	7.640	7.680	7.720	7.760	7.800	7.840	7.880	7.920	7.960
	9	8.550	8.595	8.640	8.685	8.730	8.775	8.820	8.865	8.910	8.955
20	1	1.000	1.005	1.010	1.015	1.020	1.025	1.030	1.035	1.040	1.045
	2	2.000	2.010	2.020	2.030	2.040	2.050	2.060	2.070	2.080	2.090
	3	3.000	3.015	3.030	3.045	3.060	3.075	3.090	3.105	3.120	3.135
	4	4.000	4.020	4.040	4.060	4.080	4.100	4.120	4.140	4.160	4.180
	5	5.000	5.025	5.050	5.075	5.100	5.125	5.150	5.175	5.200	5.225
	6	6.000	6.030	6.060	6.090	6.120	6.150	6.180	6.210	6.240	6.270
	7	7.000	7.035	7.070	7.105	7.140	7.175	7.210	7.245	7.280	7.315
	8	8.000	8.040	8.080	8.120	8.160	8.200	8.240	8.280	8.320	8.360
	9	9.000	9.045	9.090	9.135	9.180	9.225	9.270	9.315	9.360	9.405
21	1	1.050	1.055	1.060	1.065	1.070	1.075	1.080	1.085	1.090	1.095
	2	2.100	2.110	2.120	2.130	2.140	2.150	2.160	2.170	2.180	2.190
	3	3.150	3.165	3.180	3.195	3.210	3.225	3.240	3.255	3.270	3.285
	4	4.200	4.220	4.240	4.260	4.280	4.300	4.320	4.340	4.360	4.380
	5	5.250	5.275	5.300	5.325	5.350	5.375	5.400	5.425	5.450	5.475
	6	6.300	6.330	6.360	6.390	6.420	6.450	6.480	6.510	6.540	6.570
	7	7.350	7.385	7.420	7.455	7.490	7.525	7.560	7.595	7.630	7.665
	8	8.400	8.440	8.480	8.520	8.560	8.600	8.640	8.680	8.720	8.760
	9	9.450	9.495	9.540	9.585	9.630	9.675	9.720	9.765	9.810	9.855
22	1	1.100	1.105	1.110	1.115	1.120	1.125	1.130	1.135	1.140	1.145
	2	2.200	2.210	2.220	2.230	2.240	2.250	2.260	2.270	2.280	2.290
	3	3.300	3.315	3.330	3.345	3.360	3.375	3.390	3.405	3.420	3.435
	4	4.400	4.420	4.440	4.460	4.480	4.500	4.520	4.540	4.560	4.580
	5	5.500	5.525	5.550	5.575	5.600	5.625	5.650	5.675	5.700	5.725
	6	6.600	6.630	6.660	6.690	6.720	6.750	6.780	6.810	6.840	6.870
	7	7.700	7.735	7.770	7.805	7.840	7.875	7.910	7.945	7.980	8.015
	8	8.800	8.840	8.880	8.920	8.960	9.000	9.040	9.080	9.120	9.160
	9	9.900	9.945	9.990	10.035	10.080	10.125	10.170	10.215	10.260	10.305
23	1	1.150	1.155	1.160	1.165	1.170	1.175	1.180	1.185	1.190	1.195
	2	2.300	2.310	2.320	2.330	2.340	2.350	2.360	2.370	2.380	2.390
	3	3.450	3.465	3.480	3.495	3.510	3.525	3.540	3.555	3.570	3.585
	4	4.600	4.620	4.640	4.660	4.680	4.700	4.720	4.740	4.760	4.780
	5	5.750	5.775	5.800	5.825	5.850	5.875	5.900	5.925	5.950	5.975
	6	6.900	6.930	6.960	6.990	7.020	7.050	7.080	7.110	7.140	7.170
	7	8.050	8.085	8.120	8.155	8.190	8.225	8.260	8.295	8.330	8.365
	8	9.200	9.240	9.280	9.320	9.360	9.400	9.440	9.480	9.520	9.560
	9	10.350	10.395	10.440	10.485	10.530	10.575	10.620	10.665	10.710	10.755
24	1	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235	1.240	1.245
	2	2.400	2.410	2.420	2.430	2.440	2.450	2.460	2.470	2.480	2.490
	3	3.600	3.615	3.630	3.645	3.660	3.675	3.690	3.705	3.720	3.735
	4	4.800	4.820	4.840	4.860	4.880	4.900	4.920	4.940	4.960	4.980
	5	6.000	6.025	6.050	6.075	6.100	6.125	6.150	6.175	6.200	6.225
	6	7.200	7.230	7.260	7.290	7.320	7.350	7.380	7.410	7.440	7.470
	7	8.400	8.435	8.470	8.505	8.540	8.575	8.610	8.645	8.680	8.715
	8	9.600	9.640	9.680	9.720	9.760	9.800	9.840	9.880	9.920	9.960
	9	10.800	10.845	10.890	10.935	10.980	11.025	11.070	11.115	11.160	11.205

TABLE III.—Continued

<i>D</i>	<i>C</i>	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
25	1	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285	1.290	1.295
	2	2.500	2.510	2.520	2.530	2.540	2.550	2.560	2.570	2.580	2.590
	3	3.750	3.765	3.780	3.795	3.810	3.825	3.840	3.855	3.870	3.885
	4	5.000	5.020	5.040	5.060	5.080	5.100	5.120	5.140	5.160	5.180
	5	6.250	6.275	6.300	6.325	6.350	6.375	6.400	6.425	6.450	6.475
	6	7.500	7.530	7.560	7.590	7.620	7.650	7.680	7.710	7.740	7.770
	7	8.750	8.785	8.820	8.855	8.890	8.925	8.960	8.995	9.030	9.065
	8	10.000	10.040	10.080	10.120	10.160	10.200	10.240	10.280	10.320	10.360
	9	11.250	11.295	11.340	11.385	11.430	11.475	11.520	11.565	11.610	11.655
26	1	1.300	1.305	1.310	1.315	1.320	1.325	1.330	1.335	1.340	1.345
	2	2.600	2.610	2.620	2.630	2.640	2.650	2.660	2.670	2.680	2.690
	3	3.900	3.915	3.930	3.945	3.960	3.975	3.990	4.005	4.020	4.035
	4	5.200	5.220	5.240	5.260	5.280	5.300	5.320	5.340	5.360	5.380
	5	6.500	6.525	6.550	6.575	6.600	6.625	6.650	6.675	6.700	6.725
	6	7.800	7.830	7.860	7.890	7.920	7.950	7.980	8.010	8.040	8.070
	7	9.100	9.135	9.170	9.205	9.240	9.275	9.310	9.345	9.380	9.415
	8	10.400	10.440	10.480	10.520	10.560	10.600	10.640	10.680	10.720	10.760
	9	11.700	11.745	11.790	11.835	11.880	11.925	11.970	12.015	12.060	12.105
27	1	1.350	1.355	1.360	1.365	1.370	1.375	1.380	1.385	1.390	1.395
	2	2.700	2.710	2.720	2.730	2.740	2.750	2.760	2.770	2.780	2.790
	3	4.050	4.065	4.080	4.095	4.110	4.125	4.140	4.155	4.170	4.185
	4	5.400	5.420	5.440	5.460	5.480	5.500	5.520	5.540	5.560	5.580
	5	6.750	6.775	6.800	6.825	6.850	6.875	6.900	6.925	6.950	6.975
	6	8.100	8.130	8.160	8.190	8.220	8.250	8.280	8.310	8.340	8.370
	7	9.450	9.485	9.520	9.555	9.590	9.625	9.660	9.695	9.730	9.765
	8	10.800	10.840	10.880	10.920	10.960	11.000	11.040	11.080	11.120	11.160
	9	12.150	12.195	12.240	12.285	12.330	12.375	12.420	12.465	12.510	12.555
28	1	1.400	1.405	1.410	1.415	1.420	1.425	1.430	1.435	1.440	1.445
	2	2.800	2.810	2.820	2.830	2.840	2.850	2.860	2.870	2.880	2.890
	3	4.200	4.215	4.230	4.245	4.260	4.275	4.290	4.305	4.320	4.335
	4	5.600	5.620	5.640	5.660	5.680	5.700	5.720	5.740	5.760	5.780
	5	7.000	7.025	7.050	7.075	7.100	7.125	7.150	7.175	7.200	7.225
	6	8.400	8.430	8.460	8.490	8.520	8.550	8.580	8.610	8.640	8.670
	7	9.800	9.835	9.870	9.905	9.940	9.975	10.010	10.045	10.080	10.115
	8	11.200	11.240	11.280	11.320	11.360	11.400	11.440	11.480	11.520	11.560
	9	12.600	12.645	12.690	12.735	12.780	12.825	12.870	12.915	12.960	13.005
29	1	1.450	1.455	1.460	1.465	1.470	1.475	1.480	1.485	1.490	1.495
	2	2.900	2.910	2.920	2.930	2.940	2.950	2.960	2.970	2.980	2.990
	3	4.350	4.365	4.380	4.395	4.410	4.425	4.440	4.455	4.470	4.485
	4	5.800	5.820	5.840	5.860	5.880	5.900	5.920	5.940	5.960	5.980
	5	7.250	7.275	7.300	7.325	7.350	7.375	7.400	7.425	7.450	7.475
	6	8.700	8.730	8.760	8.790	8.820	8.850	8.880	8.910	8.940	8.970
	7	10.150	10.185	10.220	10.255	10.290	10.325	10.360	10.395	10.430	10.465
	8	11.600	11.640	11.680	11.720	11.760	11.800	11.840	11.880	11.920	11.960
	9	13.050	13.095	13.140	13.185	13.230	13.275	13.320	13.365	13.410	13.455
30	1	1.500	1.505	1.510	1.515	1.520	1.525	1.530	1.535	1.540	1.545
	2	3.000	3.010	3.020	3.030	3.040	3.050	3.060	3.070	3.080	3.090
	3	4.500	4.515	4.530	4.545	4.560	4.575	4.590	4.605	4.620	4.635
	4	6.000	6.020	6.040	6.060	6.080	6.100	6.120	6.140	6.160	6.180
	5	7.500	7.525	7.550	7.575	7.600	7.625	7.650	7.675	7.700	7.725
	6	9.000	9.030	9.060	9.090	9.120	9.150	9.180	9.210	9.240	9.270
	7	10.500	10.535	10.570	10.605	10.640	10.675	10.710	10.745	10.780	10.815
	8	12.000	12.040	12.080	12.120	12.160	12.200	12.240	12.280	12.320	12.360
	9	13.500	13.545	13.590	13.635	13.680	13.725	13.770	13.815	13.860	13.905

\* The integral parts of the dividends, *D*, are shown in the first column, and the fractional parts at the top of the page. The constant factors, *C*, are shown in the second column, and products are opposite them in each case, under the appropriate fractional part of the dividend. Values from 0.001 to 1000 times as great are obtained by moving the decimal point.

TABLE IV

VALUES FOR DIVIDENDS DIVIDED BY 25 AND MULTIPLIED BY 1 TO 9  
WHEN COLORIMETER READINGS RANGE FROM 16.0 TO 37.9

D	C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
16	1	0.640	0.644	0.648	0.652	0.656	0.660	0.664	0.668	0.672	0.676
	2	1.280	1.288	1.296	1.304	1.312	1.320	1.328	1.336	1.344	1.352
	3	1.920	1.932	1.944	1.956	1.968	1.980	1.992	2.004	2.016	2.028
	4	2.560	2.576	2.592	2.608	2.624	2.640	2.656	2.672	2.688	2.704
	5	3.200	3.220	3.240	3.260	3.280	3.300	3.320	3.340	3.360	3.380
	6	3.840	3.864	3.888	3.912	3.936	3.960	3.984	4.008	4.032	4.056
	7	4.480	4.508	4.536	4.564	4.592	4.620	4.648	4.676	4.704	4.732
	8	5.120	5.152	5.184	5.216	5.248	5.280	5.312	5.344	5.376	5.408
	9	5.760	5.796	5.832	5.868	5.904	5.940	5.976	6.012	6.048	6.084
17	1	0.680	0.684	0.688	0.692	0.696	0.700	0.704	0.708	0.712	0.716
	2	1.360	1.368	1.376	1.384	1.392	1.400	1.408	1.416	1.424	1.432
	3	2.040	2.052	2.064	2.076	2.088	2.100	2.112	2.124	2.136	2.148
	4	2.720	2.736	2.752	2.768	2.784	2.800	2.816	2.832	2.848	2.864
	5	3.400	3.420	3.440	3.460	3.480	3.500	3.520	3.540	3.560	3.580
	6	4.080	4.104	4.128	4.152	4.176	4.200	4.224	4.248	4.272	4.296
	7	4.760	4.788	4.816	4.844	4.872	4.900	4.928	4.956	4.984	5.012
	8	5.440	5.472	5.504	5.536	5.568	5.600	5.632	5.664	5.696	5.728
	9	6.120	6.156	6.192	6.228	6.264	6.300	6.336	6.372	6.408	6.444
18	1	0.720	0.724	0.728	0.732	0.736	0.740	0.744	0.748	0.752	0.756
	2	1.440	1.448	1.456	1.464	1.472	1.480	1.488	1.496	1.504	1.512
	3	2.160	2.172	2.184	2.196	2.208	2.220	2.232	2.244	2.256	2.268
	4	2.880	2.896	2.912	2.928	2.944	2.960	2.976	2.992	3.008	3.024
	5	3.600	3.620	3.640	3.660	3.680	3.700	3.720	3.740	3.760	3.780
	6	4.320	4.344	4.368	4.392	4.416	4.440	4.464	4.488	4.512	4.536
	7	5.040	5.068	5.096	5.124	5.152	5.180	5.208	5.236	5.264	5.292
	8	5.760	5.792	5.824	5.856	5.888	5.920	5.952	5.984	6.016	6.048
	9	6.480	6.516	6.552	6.588	6.624	6.660	6.696	6.732	6.768	6.804
19	1	0.760	0.764	0.768	0.772	0.776	0.780	0.784	0.788	0.792	0.796
	2	1.520	1.528	1.536	1.544	1.552	1.560	1.568	1.576	1.584	1.592
	3	2.280	2.292	2.304	2.316	2.328	2.340	2.352	2.364	2.376	2.388
	4	3.040	3.056	3.072	3.088	3.104	3.120	3.136	3.152	3.168	3.184
	5	3.800	3.820	3.840	3.860	3.880	3.900	3.920	3.940	3.960	3.980
	6	4.560	4.584	4.608	4.632	4.656	4.680	4.704	4.728	4.752	4.776
	7	5.320	5.348	5.376	5.404	5.432	5.460	5.488	5.516	5.544	5.572
	8	6.080	6.112	6.144	6.176	6.208	6.240	6.272	6.304	6.336	6.368
	9	6.840	6.876	6.912	6.948	6.984	7.020	7.056	7.092	7.128	7.164
20	1	0.800	0.804	0.808	0.812	0.816	0.820	0.824	0.828	0.832	0.836
	2	1.600	1.608	1.616	1.624	1.632	1.640	1.648	1.656	1.664	1.672
	3	2.400	2.412	2.424	2.436	2.448	2.460	2.472	2.484	2.496	2.508
	4	3.200	3.216	3.232	3.248	3.264	3.280	3.296	3.312	3.328	3.344
	5	4.000	4.020	4.040	4.060	4.080	4.100	4.120	4.140	4.160	4.180
	6	4.800	4.824	4.848	4.872	4.896	4.920	4.944	4.968	4.992	5.016
	7	5.600	5.628	5.656	5.684	5.712	5.740	5.768	5.796	5.824	5.852
	8	6.400	6.432	6.464	6.496	6.528	6.560	6.592	6.624	6.656	6.688
	9	7.200	7.236	7.272	7.308	7.344	7.380	7.416	7.452	7.488	7.524

TABLE IV—Continued

D	C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
21	1	0.840	0.844	0.848	0.852	0.856	0.860	0.864	0.868	0.872	0.876
	2	1.680	1.688	1.696	1.704	1.712	1.720	1.728	1.736	1.744	1.752
	3	2.520	2.532	2.544	2.556	2.568	2.580	2.592	2.604	2.616	2.628
	4	3.360	3.376	3.392	3.408	3.424	3.440	3.456	3.472	3.488	3.504
	5	4.200	4.220	4.240	4.260	4.280	4.300	4.320	4.340	4.360	4.380
	6	5.040	5.064	5.088	5.112	5.136	5.160	5.184	5.208	5.232	5.256
	7	5.880	5.908	5.936	5.964	5.992	6.020	6.048	6.076	6.104	6.132
	8	6.720	6.752	6.784	6.816	6.848	6.880	6.912	6.944	6.976	7.008
	9	7.560	7.596	7.632	7.668	7.704	7.740	7.776	7.812	7.848	7.884
22	1	0.880	0.884	0.888	0.892	0.896	0.900	0.904	0.908	0.912	0.916
	2	1.760	1.768	1.776	1.784	1.792	1.800	1.808	1.816	1.824	1.832
	3	2.640	2.652	2.664	2.676	2.688	2.700	2.712	2.724	2.736	2.748
	4	3.520	3.536	3.552	3.568	3.584	3.600	3.616	3.632	3.548	3.664
	5	4.400	4.420	4.440	4.460	4.480	4.500	4.520	4.540	4.560	4.580
	6	5.280	5.304	5.328	5.352	5.376	5.400	5.424	5.448	5.472	5.496
	7	6.160	6.188	6.216	6.244	6.272	6.300	6.328	6.356	6.384	6.412
	8	7.040	7.072	7.104	7.136	7.168	7.200	7.232	7.264	7.296	7.328
	9	7.920	7.956	7.992	8.028	8.064	8.100	8.136	8.172	8.208	8.244
23	1	0.920	0.924	0.928	0.932	0.936	0.940	0.944	0.948	0.952	0.956
	2	1.840	1.848	1.856	1.864	1.872	1.880	1.888	1.896	1.904	1.912
	3	2.760	2.772	2.784	2.796	2.808	2.820	2.832	2.844	2.856	2.868
	4	3.680	3.696	3.712	3.728	3.744	3.760	3.776	3.792	3.808	3.824
	5	4.600	4.620	4.640	4.660	4.680	4.700	4.720	4.740	4.760	4.780
	6	5.520	5.544	5.568	5.592	5.616	5.640	5.664	5.688	5.712	5.736
	7	6.440	6.468	6.496	6.524	6.552	6.580	6.608	6.636	6.664	6.692
	8	7.360	7.392	7.424	7.456	7.488	7.520	7.552	7.584	7.616	7.648
	9	8.280	8.316	8.352	8.388	8.424	8.460	8.496	8.532	8.568	8.604
24	1	0.960	0.964	0.968	0.972	0.976	0.980	0.984	0.988	0.992	0.996
	2	1.920	1.928	1.936	1.944	1.952	1.960	1.968	1.976	1.984	1.992
	3	2.880	2.892	2.904	2.916	2.928	2.940	2.952	2.964	2.976	2.988
	4	3.840	3.856	3.872	3.888	3.904	3.920	3.936	3.952	3.968	3.984
	5	4.800	4.820	4.840	4.860	4.880	4.900	4.920	4.940	4.960	4.980
	6	5.760	5.784	5.808	5.832	5.856	5.880	5.904	5.928	5.952	5.976
	7	6.720	6.748	6.776	6.804	6.832	6.860	6.888	6.916	6.944	6.972
	8	7.680	7.712	7.744	7.776	7.808	7.840	7.872	7.904	7.936	7.968
	9	8.640	8.676	8.712	8.748	8.784	8.820	8.856	8.892	8.928	8.964
25	1	1.000	1.004	1.008	1.012	1.016	1.020	1.024	1.028	1.032	1.036
	2	2.000	2.008	2.016	2.024	2.032	2.040	2.048	2.056	2.064	2.072
	3	3.000	3.012	3.024	3.036	3.048	3.060	3.072	3.084	3.096	3.108
	4	4.000	4.016	4.032	4.048	4.064	4.080	4.096	4.112	4.128	4.144
	5	5.000	5.020	5.040	5.060	5.080	5.100	5.120	5.140	5.160	5.180
	6	6.000	6.024	6.048	6.072	6.096	6.120	6.144	6.168	6.192	6.216
	7	7.000	7.028	7.056	7.084	7.112	7.140	7.168	7.196	7.224	7.252
	8	8.000	8.032	8.064	8.096	8.128	8.160	8.192	8.224	8.256	8.288
	9	9.000	9.036	9.072	9.108	9.144	9.180	9.216	9.252	9.288	9.324
26	1	1.040	1.044	1.048	1.052	1.056	1.060	1.064	1.068	1.072	1.076
	2	2.080	2.088	2.096	2.104	2.112	2.120	2.128	2.136	2.144	2.152
	3	3.120	3.132	3.144	3.156	3.168	3.180	3.192	3.204	3.216	3.228
	4	4.160	4.176	4.192	4.208	4.224	4.240	4.256	4.272	4.288	4.304
	5	5.200	5.220	5.240	5.260	5.280	5.300	5.320	5.340	5.360	5.380
	6	6.240	6.264	6.288	6.312	6.336	6.360	6.384	6.408	6.432	6.456
	7	7.280	7.308	7.336	7.364	7.392	7.420	7.448	7.476	7.504	7.532
	8	8.320	8.352	8.384	8.416	8.448	8.480	8.512	8.544	8.576	8.608
	9	9.360	9.396	9.432	9.468	9.504	9.540	9.576	9.612	9.648	9.684

TABLE IV—Continued

<i>D</i>	<i>C</i>	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
27	1	1.080	1.084	1.088	1.092	1.096	1.100	1.104	1.108	1.112	1.116
	2	2.160	2.168	2.176	2.184	2.192	2.200	2.208	2.216	2.224	2.232
	3	3.240	3.252	3.264	3.276	3.288	3.300	3.312	3.324	3.336	3.348
	4	4.320	4.336	4.352	4.368	4.384	4.400	4.416	4.432	4.448	4.464
	5	5.400	5.420	5.440	5.460	5.480	5.500	5.520	5.540	5.560	5.580
	6	6.480	6.504	6.528	6.552	6.576	6.600	6.624	6.648	6.672	6.696
	7	7.560	7.588	7.616	7.644	7.672	7.700	7.728	7.756	7.784	7.812
	8	8.640	8.762	8.704	8.736	8.768	8.800	8.832	8.864	8.896	8.928
	9	9.720	9.756	9.792	9.828	9.864	9.900	9.936	9.972	10.008	10.044
28	1	1.120	1.124	1.128	1.132	1.136	1.140	1.144	1.148	1.152	1.156
	2	2.240	2.248	2.256	2.264	2.272	2.280	2.288	2.296	2.304	2.312
	3	3.360	3.372	3.384	3.396	3.408	3.420	3.432	3.444	3.456	3.468
	4	4.480	4.496	4.512	4.528	4.544	4.560	4.576	4.592	4.608	4.624
	5	5.600	5.620	5.640	5.660	5.680	5.700	5.720	5.740	5.760	5.780
	6	6.720	6.744	6.768	6.792	6.816	6.840	6.864	6.888	6.912	6.936
	7	7.840	7.868	7.896	7.924	7.952	7.980	8.008	8.036	8.064	8.092
	8	8.960	8.992	9.024	9.056	9.088	9.120	9.152	9.184	9.216	9.248
	9	10.080	10.116	10.152	10.188	10.224	10.260	10.296	10.332	10.368	10.404
29	1	1.160	1.164	1.168	1.172	1.176	1.180	1.184	1.188	1.192	1.196
	2	2.320	2.328	2.336	2.344	2.352	2.360	2.368	2.376	2.384	2.392
	3	3.480	3.492	3.504	3.516	3.528	3.540	3.552	3.564	3.576	3.588
	4	4.640	4.656	4.672	4.688	4.704	4.720	4.736	4.752	4.768	4.784
	5	5.800	5.820	5.840	5.860	5.880	5.900	5.920	5.940	5.960	5.980
	6	6.960	6.984	7.008	7.032	7.056	7.080	7.104	7.128	7.152	7.176
	7	8.120	8.148	8.176	8.204	8.232	8.260	8.288	8.316	8.344	8.372
	8	9.280	9.312	9.344	9.376	9.408	9.440	9.472	9.504	9.536	9.568
	9	10.440	10.476	10.512	10.548	10.584	10.620	10.656	10.692	10.728	10.764
30	1	1.200	1.204	1.208	1.212	1.216	1.220	1.224	1.228	1.232	1.236
	2	2.400	2.408	2.416	2.424	2.432	2.440	2.448	2.456	2.464	2.472
	3	3.600	3.612	3.624	3.636	3.648	3.660	3.672	3.684	3.696	3.708
	4	4.800	4.816	4.832	4.848	4.864	4.880	4.896	4.912	4.928	4.944
	5	6.000	6.020	6.040	6.060	6.080	6.100	6.120	6.140	6.160	6.180
	6	7.200	7.224	7.248	7.272	7.296	7.320	7.344	7.368	7.392	7.416
	7	8.400	8.428	8.456	8.484	8.512	8.540	8.568	8.596	8.624	8.652
	8	9.600	9.632	9.664	9.696	9.728	9.760	9.792	9.824	9.856	9.888
	9	10.800	10.836	10.872	10.908	10.944	10.980	11.016	11.052	11.088	11.124
31	1	1.240	1.244	1.248	1.252	1.256	1.260	1.264	1.268	1.272	1.276
	2	2.480	2.488	2.496	2.504	2.512	2.520	2.528	2.536	2.544	2.552
	3	3.720	3.732	3.744	3.756	3.768	3.780	3.792	3.804	3.816	3.828
	4	4.960	4.976	4.992	5.008	5.024	5.040	5.056	5.072	5.088	5.104
	5	6.200	6.220	6.240	6.260	6.280	6.300	6.320	6.340	6.360	6.380
	6	7.440	7.464	7.488	7.512	7.536	7.560	7.584	7.608	7.632	7.656
	7	8.680	8.708	8.736	8.764	8.792	8.820	8.848	8.876	8.904	8.932
	8	9.920	9.952	9.984	10.016	10.048	10.080	10.112	10.144	10.176	10.208
	9	11.160	11.196	11.232	11.268	11.304	11.340	11.376	11.412	11.448	11.484
32	1	1.280	1.284	1.288	1.292	1.296	1.300	1.304	1.308	1.312	1.316
	2	2.560	2.568	2.576	2.584	2.592	2.600	2.608	2.616	2.624	2.632
	3	3.840	3.852	3.864	3.876	3.888	3.900	3.912	3.924	3.936	3.948
	4	5.120	5.136	5.152	5.168	5.184	5.200	5.216	5.232	5.248	5.264
	5	6.400	6.420	6.440	6.460	6.480	6.500	6.520	6.540	6.560	6.580
	6	7.680	7.704	7.728	7.752	7.776	7.800	7.824	7.848	7.872	7.896
	7	8.960	8.988	9.016	9.044	9.072	9.100	9.128	9.156	9.184	9.212
	8	10.240	10.272	10.304	10.336	10.368	10.400	10.432	10.464	10.496	10.528
	9	11.520	11.556	11.592	11.628	11.664	11.700	11.736	11.772	11.808	11.844

TABLE IV—Continued

<i>D</i>	<i>C</i>	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
33	1	1.320	1.324	1.328	1.332	1.336	1.340	1.344	1.348	1.352	1.356
	2	2.640	2.648	2.656	2.664	2.672	2.680	2.688	2.696	2.704	2.712
	3	3.960	3.972	3.984	3.996	4.008	4.020	4.032	4.044	4.056	4.068
	4	5.280	5.296	5.312	5.328	5.344	5.360	5.376	5.392	5.408	5.424
	5	6.600	6.620	6.640	6.660	6.680	6.700	6.720	6.740	6.760	6.780
	6	7.920	7.944	7.968	7.992	8.016	8.040	8.064	8.088	8.112	8.136
	7	9.240	9.268	9.296	9.324	9.352	9.380	9.408	9.436	9.464	9.492
	8	10.560	10.592	10.624	10.656	10.688	10.720	10.752	10.784	10.816	10.848
	9	11.880	11.916	11.952	11.988	12.024	12.060	12.096	12.132	12.168	12.204
34	1	1.360	1.364	1.368	1.372	1.376	1.380	1.384	1.388	1.392	1.396
	2	2.720	2.728	2.736	2.744	2.752	2.760	2.768	2.776	2.784	2.792
	3	4.080	4.092	4.104	4.116	4.128	4.140	4.152	4.164	4.176	4.188
	4	5.440	5.456	5.472	5.488	5.504	5.520	5.536	5.552	5.568	5.584
	5	6.800	6.820	6.840	6.860	6.880	6.900	6.920	6.940	6.960	6.980
	6	8.160	8.184	8.208	8.232	8.256	8.280	8.304	8.328	8.352	8.376
	7	9.520	9.548	9.576	9.604	9.632	9.660	9.688	9.716	9.744	9.772
	8	10.880	10.912	10.944	10.976	11.008	11.040	11.072	11.104	11.136	11.168
	9	12.240	12.276	12.312	12.348	12.384	12.420	12.456	12.492	12.528	12.564
35	1	1.400	1.404	1.408	1.412	1.416	1.420	1.424	1.428	1.432	1.436
	2	2.800	2.808	2.816	2.824	2.832	2.840	2.848	2.856	2.864	2.872
	3	4.200	4.212	4.224	4.236	4.248	4.260	4.272	4.284	4.296	4.308
	4	5.600	5.616	5.632	5.648	5.664	5.680	5.696	5.712	5.728	5.744
	5	7.000	7.020	7.040	7.060	7.080	7.100	7.120	7.140	7.160	7.180
	6	8.400	8.424	8.448	8.472	8.496	8.520	8.544	8.568	8.592	8.616
	7	9.800	9.828	9.856	9.884	9.912	9.940	9.968	9.996	10.024	10.052
	8	11.200	11.232	11.264	11.296	11.328	11.360	11.392	11.424	11.456	11.488
	9	12.600	12.636	12.672	12.708	12.744	12.780	12.816	12.852	12.888	12.924
36	1	1.440	1.444	1.448	1.452	1.456	1.460	1.464	1.468	1.472	1.476
	2	2.880	2.888	2.896	2.904	2.912	2.920	2.928	2.936	2.944	2.952
	3	4.320	4.332	4.344	4.356	4.368	4.380	4.392	4.404	4.416	4.428
	4	5.760	5.776	5.792	5.808	5.824	5.840	5.856	5.872	5.888	5.904
	5	7.200	7.220	7.240	7.260	7.280	7.300	7.320	7.340	7.360	7.380
	6	8.640	8.664	8.688	8.712	8.736	8.760	8.784	8.808	8.832	8.856
	7	10.080	10.108	10.136	10.164	10.192	10.220	10.248	10.276	10.304	10.332
	8	11.520	11.552	11.584	11.616	11.648	11.680	11.712	11.744	11.776	11.808
	9	12.960	12.996	13.032	13.068	13.104	13.140	13.176	13.212	13.248	13.284
37	1	1.480	1.484	1.488	1.492	1.496	1.500	1.504	1.508	1.512	1.516
	2	2.960	2.968	2.976	2.984	2.992	3.000	3.008	3.016	3.024	3.032
	3	4.440	4.452	4.464	4.476	4.488	4.500	4.512	4.524	4.536	4.548
	4	5.920	5.936	5.952	5.968	5.984	6.000	6.016	6.032	6.048	6.064
	5	7.400	7.420	7.440	7.460	7.480	7.500	7.520	7.540	7.560	7.580
	6	8.880	8.904	8.928	8.952	8.976	9.000	9.024	9.048	9.072	9.096
	7	10.360	10.388	10.416	10.444	10.472	10.500	10.528	10.556	10.584	10.612
	8	11.840	11.872	11.904	11.936	11.968	12.000	12.032	12.064	12.096	12.128
	9	13.320	13.356	13.392	13.428	13.464	13.500	13.536	13.572	13.608	13.644

## CHAPTER IV

### COLORIMETER CALIBRATION AND CORRECTION CURVES <sup>1</sup>

BEER'S law, the basis of colorimetric analysis, holds only where the color formations in the solutions to be compared are perfectly formed and stable or are the same in both solutions. In actual analytical work the color formation is often incomplete or unstable and the results then do not obey Beer's law. In other words, change in the amount of color or light means a change in the amount of reaction product. The customary method of overcoming this error is the use of a calibration or a correction curve, prepared experimentally with solutions of known concentrations. Such curves have appeared in the literature and are of great value in interpreting the results of analytical work and in calling attention to the magnitude of the error, but they must not be taken literally by the reader, as Wright<sup>2</sup> has recently pointed out. Whether the different degrees of error, i.e., variations in the curves, are due to personal variation in matching colors that do not exactly match in tint, to differences among colorimeters, or to unconscious differences in technique, would be difficult to decide. It is, however, a simple matter to prepare such a curve, and if the average deviation from the mean at each concentration be calculated, a fair notion of the probable error of any reading may be had. The author, therefore, urges that a calibration or a correction curve be obtained for each method deviating from Beer's law and that such graphs be not used by others, without verification.

Figures 36<sup>a</sup> and 37 illustrate two ways of constructing colorimeter curves. The data from which the curves are constructed are given in Table V. Column 1 of the table gives the readings of the standards when the unknown solutions are set at 20 mm. This procedure eliminates all but a simple mental calculation when the solution follows Beer's law, and when the data are plotted they yield a straight line

<sup>1</sup> This chapter was written with the assistance of Philip Adolph Kober.

<sup>2</sup> J. Biol. Chem., **71**, 209 (1926-27).

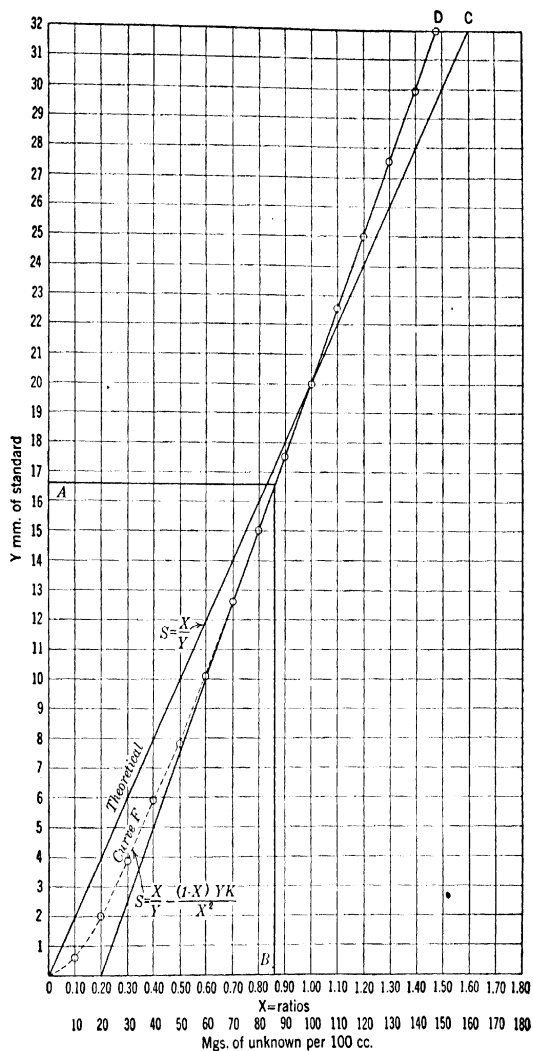


FIG. 36 \*

\* In the equations  $\frac{X}{Y}$  should read  $\frac{Y}{X}$ .



instead of a curve. (Figs. 36 and 37.) Column 2 gives the ratios of the readings of the standard to the readings of the unknowns; column 3 contains the concentrations of the unknown in milligrams per 100 cc. obtained in the usual way from these readings, i.e., assuming Beer's law is obeyed; while column 5 gives the actual concentrations to which the latter readings correspond. In column 4 the corrections are given.

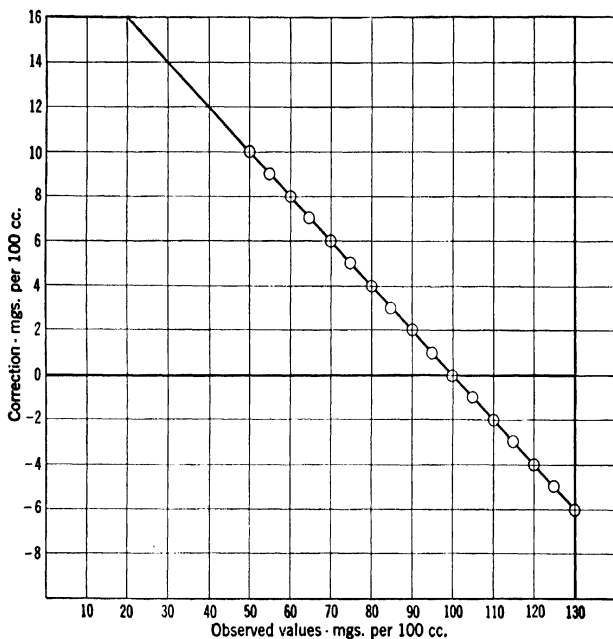


FIG. 37.

In Fig. 36 the "Actual Curve," *D*, is obtained by plotting the colorimetric readings as abscissas against the actual concentrations as ordinates. Theoretically the points should fall on the "Theoretical Curve," but actually such is sometimes not the case, especially if the concentrations of the standard and unknown solutions differ widely.

An example will illustrate the use of the colorimeter curve (curve *D*): Suppose the ratio of the height of the standard to the height of the unknown is found to be 0.83, i.e., the height of the standard was 16.6

mm. when it matched the unknown kept at 20.0 mm. Find the amount of unknown per 100 cc. Read 16.6 on the  $y$ -axis, follow the horizontal line  $A$  till it intersects the "actual curve," then follow the vertical line  $B$  till it cuts the  $x$ -axis, and read the point of intersection. In this case, 86.0 mg. of unknown per 100 cc. is obtained. These curves are drawn on a small scale for the sake of convenience, but in actual work they may be constructed to any scale, sufficiently large to obtain any desired accuracy.

TABLE V

Colorimeter Reading Standard (Unknown set at 20)		Observed		Substance, Mg. per 100 cc.				Cor- rec- tion, Mg. per 100 cc.
(K = -0.244)	Readings, Actual	Ratio of H. of S. H. of Un.	Concentra- tion of Unknown, Mg. per 100 cc.	Correction	Actual	Observed	Actual	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(3.86)	2.50	0.125	12.50	+17.50	30	30	44	+14
	3.75	0.1875	18.75	16.25	35	35	48	+13
(5.85)	5.00	0.250	25.00	15.00	40	40	52	+12
	6.25	0.3125	31.25	13.75	45	45	56	+11
(8.18)	7.50	0.375	37.50	12.50	50	50	60	+10
	8.75	0.4375	43.75	11.25	55	55	64	+9
(10.30)	10.00	0.500	50.00	10.00	60	60	68	+8
	11.25	0.5625	56.25	8.75	65	65	72	+7
	12.50	0.625	62.50	7.50	70	70	76	+6
	13.75	0.6875	68.75	6.25	75	75	80	+5
(15.08)	15.00	0.750	75.00	5.00	80	80	84	+4
	16.25	0.8125	81.25	3.75	85	85	88	+3
	17.50	0.875	87.50	2.50	90	90	92	+2
	18.75	0.9375	93.75	1.25	95	95	96	+1
(20.00)	20.00	1.000	100.00	0.00	100	100	100	± 0
	21.25	1.0625	106.25	-1.25	105	105	104	-1
	22.50	1.125	112.50	2.50	110	110	108	-2
	23.75	1.1875	118.75	3.75	115	115	112	-3
(25.07)	25.00	1.250	125.00	5.00	120	120	116	-4
	26.25	1.3125	131.25	6.25	125	125	120	-5
(27.50)	27.50	1.375	137.50	7.50	130	130	124	-6

H. of S. = Height of Standard

H. of Un. = Height of Unknown.

TABLE VI  
DATA FOR CURVE F, FIG. 36

$S = \frac{Y}{X} - \frac{(1-X)FK}{X^2}$	$K = -0.244$	$S = \frac{Y}{X} - \frac{(1-X)FK}{X^2}$	$K = -0.244$
$X$	$Y$	$X$	$Y$
0.00	0.00	0.80	15.08
0.10	0.62	0.90	17.52
0.20	2.02	1.00	20.00
0.30	3.82	1.10	22.50
0.40	5.85	1.20	25.00
0.50	8.17	1.30	27.50
0.60	10.30	1.40	29.92
0.70	12.67	1.48	31.91

The data in Table V may also be plotted as shown in Fig. 37 in which the observed values in column 6 are plotted as abscissas against the corrections, in column 8, as ordinates.

There are other ways of graphically recording colorimetric data, but the above two figures illustrate the more common forms of colorimeter curves. It should be mentioned that in Table V the author has purposely used data which give large corrections (except near identical concentration of unknown and standard). This has been done in order to bring out more strikingly the colorimeter curve.

For precision work each color formation should be calibrated throughout the dilutions likely to be met with in actual work. This is necessary not only to eliminate any errors of the instrument or of the solvent and any idiosyncrasies of the observer, but also to check the completion of the color formation at each dilution. While the amount of color from a given quantity of colored substance is the same in accordance with Beer's law, yet the colorimetric analyst is primarily concerned not with checking Beer's law but with the production of a colored compound at different dilutions and under different conditions, and his concern is to get the maximum colored substance in each dilution. Unless the color formation is 100 per cent in each dilution, Beer's law will not hold. In many color reactions the color formation falls short of completion, particularly when the reaction takes place in dilute solutions. Quite often, due to hydrolysis, chemical change, or instability, or a number of these factors, the relatively weaker solu-

tions have less color than the stronger solutions. In all such cases the calibration of the amount of color with the amount of substance becomes necessary as a prerequisite to precision work. Heretofore it was necessary to plot either a dilution or a correction curve. See Fig. 36 (curve *D*) and Fig. 37, respectively. But one worker in one laboratory would have difficulty in comparing his curve with that of another laboratory, because heretofore no general formula was available to express the deviation from Beer's law.

TABLE VII  
DATA FOR FIG. 38

$S = \frac{Y}{X} - \frac{(1-X)YK}{X^2}$	$K = +0.244$	$S = \frac{Y}{X} - \frac{(1-X)YK}{X^2}$	$K = +0.244$
<i>X</i>	<i>Y</i>	<i>X</i>	<i>Y</i>
0.2	166.6	0.9	18.7
0.3	13.95	1.0	20.0
0.4	12.60	1.5	27.7
0.5	13.23	1.8	32.50
0.6	—	2.0	35.65
0.7	15.6	2.6	45.3
0.8	—	2.8	49.35

Now, it has been found that between certain limits, sufficiently wide for most analytical work, the nephelometric formula developed by Kober to show deviation from Rayleigh's formula will serve to express deviation from Beer's law, thus giving mathematical expression to such deviations.

In Figs. 36 and 39, lines *C* represent readings obtained when the color is proportional to the heights of solution in accordance with Beer's law. Lines *D* and *F*<sub>2</sub> represent a deviation when the color in the weaker solution is less than 100 per cent; Line *E* (Fig. 38), when the color in the stronger solution is less than 100 per cent, which may be the case in colored suspensions.

Kober's formula in its original form was  $Y = \frac{S}{X} - \frac{(1-X)SK}{X^2}$ ,

where *Y* = readings of the solution (he varied the unknown or more dilute solution), *X* = the ratio of the concentrations of the two solutions, *S* = the setting or reading of the solution kept at a constant

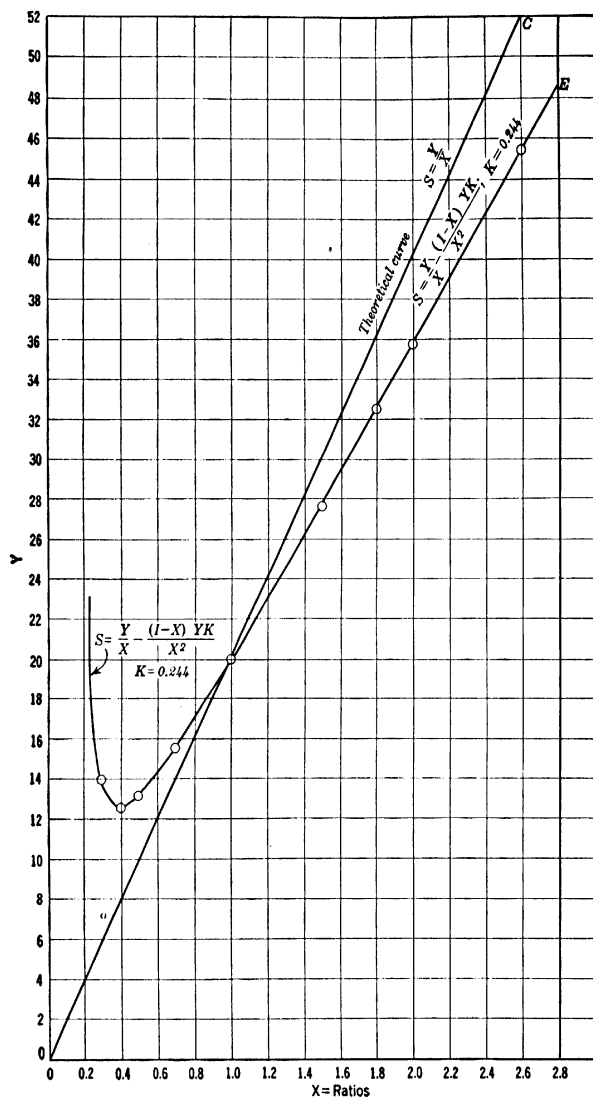


FIG. 38.

height (he kept the standard at a constant height),  $K$  = a constant for each solution. Kober then obtained a curve (Curve  $F$ , Fig. 39).

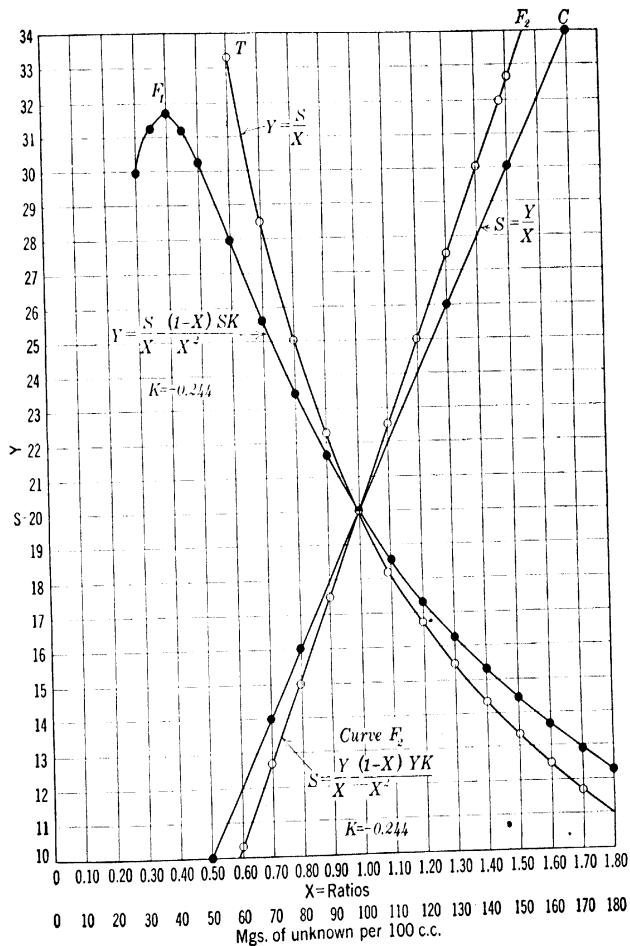


FIG. 39.

As was pointed out on page 70, by keeping the unknown at a fixed height and varying the standard, the theoretical curve following Beer's

TABLE VIII

DATA FOR CURVES  $F_1$  AND  $F_2$  IN FIG. 39

$Y = \frac{X}{S} - \frac{(1-X)SK}{X^2}$		$S = 20.0$ $K = 0.244$ $S = \frac{Y}{X} - \frac{(1-X)YK}{X^2}$	
Curve $F_1$		Curve $F_2$	
$X$	$Y$	$X$	$Y$
0.30	28.75	0.60	10.30
0.35	31.31	0.70	12.67
0.40	31.70	0.80	15.08
0.45	31.19	0.90	17.52
0.50	30.24	1.00	20.00
0.60	27.91	1.10	22.50
0.70	25.60	1.20	25.00
0.80	23.48	1.30	27.50
0.90	21.62	1.40	29.92
1.00	20.00	1.48	31.91
1.10	18.58	1.50	32.65
1.20	17.34		
1.30	16.25		
1.40	15.25		
1.50	14.42		
1.60	13.64		
1.70	12.94		
1.80	12.31		

law will then become a straight line. By using Kober's formula in the form  $S = \frac{Y}{X} - \frac{(1-X)YK}{X^2}$  his curve beyond certain points also becomes a straight line; curves  $F$ , in Figs. 36 and 39. His formula, curves  $F$ , Fig. 39, when the concentrations of the unknown are below 0.5 or 0.6 standard turn off into curves that do not apply, but in concentration above these, the line is perfectly straight. In keeping the unknown reading fixed and varying the height of the standard, it makes for greater precision, i.e., less percentage error in measuring the height of the standard if these fall above the value fixed for the unknown. In other words, when the unknown is stronger than the standard, and therefore in actual work, these deviations

would fall upon a straight line. In Fig. 40 the variations are given over a considerable scale and the constants of each line.

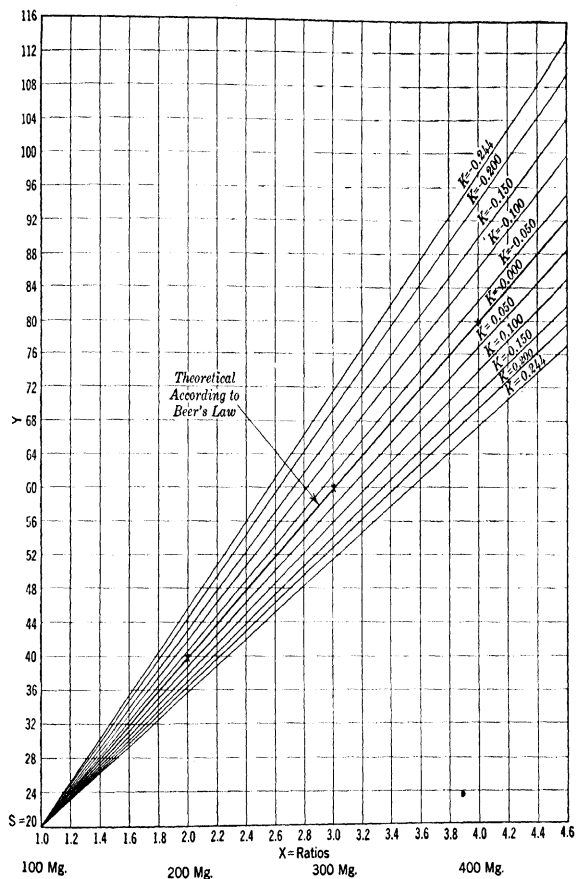


FIG. 40.

It will be observed that where the weaker solution deviates from 100 per cent of color the constant  $K$  has a negative sign, and where the



or the same deviation constant as was obtained by the first observer. In other words, the formula shows that in the two different tests by different observers the solution behaved in an identical manner.

NOTE.—A number of graph sheets have been placed in the back of the book. It will be found convenient to use these in making calibration and correction curves. Also, they are less likely to be lost here than in a loose-leaf notebook.

## CHAPTER V

### ERRORS IN COLORIMETRY

IN colorimetric analysis certain factors introducing variations and errors are frequently overlooked. Chief among these are variable sensitiveness of vision to different depths of color, the inability of many persons to judge colors accurately, and the bicolored nature of the solution.

**Variable Sensitiveness of Vision to Different Concentrations.**—Horn and his co-workers<sup>1</sup> have made an experimental study of variable sensitiveness in colorimetry. They used solutions of  $\text{CrO}_4^{--}$ ,  $\text{Cu}^{++}$ , and  $\text{Cu}(\text{NH}_3)_4^{++}$  ions and showed that with equal depths at certain definite concentrations, the comparisons in the colorimetric determinations of these ions can be made with greater ease and accuracy than at other concentrations. It is held by them that this relation is a "perfectly general one throughout colorimetry" and they suggest that "the curve of sensitiveness must be known in each colorimetric method if the method is to be used to greatest advantage and the results are not to be affected by errors of unknown magnitudes."

**Imperfect Susceptibility to Color.**<sup>2</sup>—Another cause of error in colorimetry is due to the inability of many persons to judge colors accurately. Practice will do much to enable a person to make good color comparisons, but it can never make up for a dulled or imperfect susceptibility to color. Great attention should be given to this point and an operator should test himself thoroughly for each color by matching a standard against itself in several degrees of intensity. If he fails to obtain concordant results with a certain color, it is useless for him to go further with this color. In this connection it should be noted that a person who cannot use one method with accuracy is frequently able to use another with great precision.

<sup>1</sup> Am. Chem. J., **35**, 253 (1906); *ibid.*, **36**, 195, 516 (1906); see also J. Leuba, J. Philosophy, Psychology, Scientific Methods, **4**, No. 6, 166 (1907); Yoe and Hill, J. Am. Chem. Soc. **49**, 2395 (1927).

<sup>2</sup> J. H. Yoe, J. Lab. Clin. Med., **13**, 139 (1927.)

**Fatigue and Eye Strain.**<sup>3</sup>—Care must be taken on the part of the observer to avoid fatigue and eye strain. Failure to take this precaution may introduce a serious error in otherwise excellent work. Especially is this precaution necessary when a long series of determinations is being made. In such a case, the use of a dark-room will aid greatly. Each observer must, of course, determine his capacity in this respect.

**"Fallacies in Colorimetry." Discussion by Dehn.**<sup>4</sup>—Dehn has made a study of the "fallacies in colorimetry" and gives a full discussion of them. Fortunately, most of these "fallacies" can be avoided or reduced to a satisfactory minimum and hence the reader should not be discouraged at such an array of possible "pitfalls." As a matter of fact, colorimetry offers one of the most accurate means, and in some cases the only means, of quantitatively measuring minute quantities of substances. But it is hoped that the following paragraphs will help to emphasize the importance of studying the various sources of error of each colorimetric method employed.

In most colorimetric determinations, the depth of solutions of the color standards are matched by various depths and concentrations of the solutions to be estimated; usually we have two columns of solutions of unequal length whose tints are matched to the limit of sensitiveness of the eye and whose solute-molecules are assumed to be the same in number. That the solute-molecules are not necessarily the same, not only in number but also in composition, may be concluded from studies of (1) their different optical distributions, (2) different equilibria resulting from ionization, (3) different equilibria resulting from hydrolysis, and (4) different equilibria resulting from chromoisomerization.

**Different Optical Distributions.**—In respect to different optical distributions, four cases appear—the matched solutions may have:

- (1) Equal volumes, equal depths, and equal cross-sections.
- (2) Equal volumes, equal depths, and unequal cross-sections.
- (3) Equal volumes, unequal depths, and unequal cross-sections.
- (4) Unequal volumes, unequal depths, and unequal cross-sections.

It will be shown that all of these geometrical conditions involve errors which increase from Case 1 to Case 4.

<sup>3</sup> J. H. Yoe, *loc. cit.*

<sup>4</sup> J. Am. Chem. Soc., **39**, 1392 (1917).

*Case 1.*—If, for instance, two cylinders of equal diameter are used and the solutions contained therein are viewed along the lines of the major axes, such solutions appear as truncated cones whose distant cross-sections are concentric with the near and apparently larger cross-section. That these identical figures may *appear identical*, they must be viewed at equal distance and the lines of the major axes must always intersect on the retina.<sup>5</sup> When these conditions are met with, the viewed fields are identical but never homogeneous, owing to the fact that the near and distant surfaces of the liquids intercept unequal lines which continue and intersect on the retina. Therefore, though the central portions<sup>6</sup> of the fields may be nearly homogeneous, the areas between the circumferences of the two concentric circles present fields of color rapidly decreasing toward the outer edges. The nearer the eye is to the viewed cylinder of solution, the less homogeneous will the viewed fields become; only at great distances will the viewed volumes appear approximately homogeneous and identical.

In a similar manner it may be shown that other forms of equal volumes, equal depth, and uniform cross-section involve the same errors. Never are the fields of color homogeneous, for the reason that the near and distant bounding surfaces are parallel, whereas the true lines of vision may be conceived to radiate from the retina and become intercepted between the nearer and distant parallel planes, hence are of unequal lengths.

*Case 2.*—With forms involving equal volumes, equal depths and different cross-sections, as, for instance, with cones, pyramids, sectors of spheres and their truncated forms, it can be demonstrated in a similar manner that homogeneous fields cannot be obtained; only with frustums of hollow spheres can homogeneity<sup>7</sup> be obtained, and then only when the points of vision are coincidental with the spherical centers.

<sup>5</sup> Of course, the assumption is made that the eye operates as a point of vision, whereas owing to measurable widths of pupil and retina, the eye presents an *area* of vision. This condition tends to compensate the above described variations.

<sup>6</sup> Some colorimeters are so constructed as to bring into juxtaposition the central portions of the two fields and thus to reduce to a minimum this possibility of error.

<sup>7</sup> Both homogeneity and identity of color are possible only when equal concentrations of solute molecules are contained in equal volumes of the sphere-frustum form. Even then other constant conditions are necessary, such as concentration of  $\text{OH}^-$  and  $\text{H}^+$ , temperature and intensity of illumination.

When square uniform bottles of clear glass as containing vessels are viewed at distances of many feet, the sphere-frustum formed is approximated. See J. Am. Chem. Soc., **36**, 407, 837 (1914).

*Case 3.*—When equal volumes of *unequal*<sup>8</sup> depth are viewed longitudinally, the near cross-sections being the same or different, not only are homogeneity and identity of color impossible for the above-mentioned reasons, but also two other physical conditions producing color variations are introduced.

*First, the more distant solute-molecules conceivably vary in size inversely as the square of the distance from the point of vision.* Though, of course, no individual molecule is visible, it is possible, though not demonstrable, that in near and distant viewings there may be physical differences produced in this manner by individual molecules or by molecules *en masse*. It is very probable, for this reason and for reasons given above, that the homogeneity of the viewed field is disturbed by the use of long tubes. Hence, it cannot be held that different columns of liquids of the same apparent tint contain the same number of solute-molecules.

The second physical conditions producing color variations in equal volumes of unequal depth is that *more solute-molecules*<sup>9</sup> *will constantly be behind each other* along the lines of vision in the longer columns than in the shorter columns. This physical difference probably affects the absorption of light like-colored, transparent disks of glass on a white field, when viewed singly or in columns of varied depths. The latter positions give darker shades of color, whereas the former, within cer-

<sup>8</sup> Colorimeters of the immersion type involve volumes of liquids viewed as truncated cones. They approximate the sphere-frustum form, when the depths of colored solutions are small and the distances from the eye are comparatively large. However, under any condition, the viewed fields are not homogeneous, the outer areas being darker than the central areas.

Furthermore, in colorimeters of this type, the total viewed volumes are not directly proportional to the movements of the near, trans-base bounding surfaces. If the eye is at a distance of  $a$  from this nearer surface and at a distance of  $a + b$  from the further surface, when the former, with a radius of  $r$ , is moved toward the latter a distance of  $c$ , the ratio of viewed volumes can be shown to be expressed by

$$\frac{a^2[(a + b)^3 - (a + c)^3]}{(a + c)^2[(a + b)^3 - a^3]}$$

and not expressed by the ratio of  $b \cdot c / b$ . When, for instance,  $a = 2$ ,  $b = 2$  and  $c = 1$ , the ratio of volumes is not 1 to 0.50 but 1 to 0.29 +. Now, then, if matchings of color of the entire fields are made, in this case, it will be observed an error of 42 per cent is made.

<sup>9</sup> Of course, it may be argued that the diameters of molecules are insignificant when compared to the widths of intermolecular spaces and that, consequently, this physical influence is *nil*. Though perhaps *nil* in dilute solutions, packing of solute molecules in concentrated solutions certainly produces color variations, and possibly this is the most important cause of lack of sensitiveness to changes of concentration in deep columns or in solutions already very concentrated.

tain limits of being loosely or densely crowded, give the same shade. Therefore, it is not to be expected that a solution of definite concentration viewed in a certain depth will have exactly the same shade of color as that, for instance, of a solution in double concentration and viewed in one-half of its depth.

*Case 4.*—When unequal volumes in unequal depth and different cross-sections are viewed in colorimeters, there are introduced not only all possible errors of different optical distributions, and of the two possible physical effects mentioned, but also disturbances of the three equilibria of ionization, hydrolysis, and isomerization.

**Disturbances of Equilibria Resulting from Ionization.**—In considering the effects of ionization on colored solutes, two conditions must be recognized, *viz.*, (1) the ionization of the solute-molecules themselves, and (2) the ions of the solution as affecting the solute-molecules. For example, on the one hand, the ions yielded by methyl orange must be considered, and, on the other hand, the ions of acids and alkalis affecting methyl orange molecules must be considered.

Opinion is almost universal that the one, the other, or both types of ionization affect the color of solute-molecules. However, studies<sup>10</sup> on the mass action of water on dyes, and especially on methyl orange, clearly demonstrate that *color changes are primarily, if not entirely, independent of both types of ionization.* In fact, it has been found that the *molecular* states of colored solutes are largely affected by hydrolysis, and this influence, rather than the degrees of ionization, is operative in transforming the molecules and in producing color changes. Therefore, whether or not ionization figures at all in color changes, it is certain that other factors, such as the stability of the salts of the indicator, the mass influence of water, and the effects of temperature, are far more important.<sup>11</sup>

**Disturbances of Equilibria Resulting from Hydrolysis.**—Whereas, in colorimetry, the solutions estimated as well as the solutions used for comparison are almost universally assumed to be monochromatic, experiments show that many of them are dichromatic,<sup>12</sup> that is, they are usually bicolored, like the materials used as indicators.

<sup>10</sup> J. Am. Chem. Soc., **39**, 1338, 1348 (1917).

<sup>11</sup> For the effects of temperature on color, see especially Kurbatov, J. Russ. Phys.-Chem. Soc., **39**, 456 (1909); see also J. Am. Chem. Soc., **36**, 845 (1914).

<sup>12</sup> Some may even be considered to be polychromatic. Many dyes, giving progressive changes of color from strong acid concentrations to strong alkaline concentrations, or *vice versa*, can be showed to be mixtures of only two forms.

For several years (1914-17) experimental work was carried on by Dehn and his co-workers to estimate the variations of tints of colored solutes when affected by (1) acids and alkalies, (2) heat and cold, and (3) dilution and concentration.

General knowledge in respect to the action of acids and alkalies on bicolored compounds is extensive and need not be discussed here. This important observation must be made—*at sufficiently great dilution, many, if not all, bicolored substances, whether in acidic or in alkaline solutions, are of the same tint.* Therefore, with indicators, not only are the equilibrium actions of acids and alkalies to be considered but also the mass action of water; in other words, the concentration of the solution used is not a matter of indifference in colorimetry. This was pointed out by Horn<sup>13</sup> and others, and later demonstrated by Dehn and his co-workers.<sup>14</sup>

The latter workers have shown (1) that all solutions of chromates and dichromates above 0.007 per cent concentrations are mixtures of the two in aqueous equilibria, (2) that solutions of picrates above 0.001 per cent concentrations are mixtures of tautomeric forms in aqueous equilibria, (3) that many dyes, especially those used as indicators, are changed in color by the mass action of water, and (4), finally, that all aqueous solutions of methyl orange, whether acidic or alkaline in reaction, are chromoisomeric mixtures in aqueous equilibria.

In all colorimetric methods, it is either stated or implied that Beer's law<sup>15</sup> is valid, namely, that the *tint of colored solutions is dependent upon the mass of the solute and is independent of dilution.* That this law does not hold in many cases, as recognized by numerous investigators,<sup>16</sup> is easily understood, when it is observed that water and other solvents have a transforming influence on many colored solutes. Therefore, in all colorimetric methods such influences must be recognized, the limits of dilution<sup>17</sup> must be set, and comparisons must be made under identical conditions.

<sup>13</sup> *Loc. cit.*

<sup>14</sup> J. Am. Chem. Soc., **36**, 329 (1914); *ibid.*, **39**, 1338, 1348, 1377, 1381 (1917).

<sup>15</sup> Ann. Physik. [2], **86**, 78 (1852).

<sup>16</sup> See especially Hantzsch's and Horn's papers. Also Picard, Ann., **381**, 347 (1913); Stewart and Wright, Ber., **44**, 2819 (1911).

<sup>17</sup> "It is either stated or implied in descriptions of colorimetric methods that they are to be applied to dilute solutions. This practice seems to have originated in the observation that the results of parallel determinations made in deeply colored (not very dilute) solutions do not approximate sufficiently to the average value." Horn, Am. Chem. J., **35**, 253 (1906).

**Disturbances Resulting from Chromoisomerization.**—Bicoored dyes, as methyl orange, undergo tautomeric changes when subjected to the influences of (1) acids and alkalis, (2) heat and cold, and (3) dilution and concentration. These influences have been discussed sufficiently in the foregoing. It need only be pointed out that such chromoisomerizations may be disturbed in the presence of constant concentrations of acids, alkalis and solvent, through changes of temperature. Therefore, constant temperatures, as well as constant concentrations, must be observed in all colorimetric methods.

**General Conclusions.**— From the above discussion the various possible errors to be dealt with in colorimetry may be summed up as follows:

1. Errors due to the inability of the observer to judge colors accurately.
2. Errors due to variable sensitiveness of vision to *different concentrations*.
3. Errors due to fatigue and eye strain.
4. Mechanical errors of the colorimeter.
5. Optical errors of the colorimeter.
6. Optical errors resulting from varied light.
7. Errors resulting from scale readings.
8. Errors of dilution.
9. Errors due to temperature variation.
10. Errors due to varied times of standing.
11. Errors due to varied quantities of reagents.
12. Errors due to the presence of interfering substances.
13. Errors due to variation in the hydrogen-ion concentration.
14. Errors due to the action of light.

It would seem from the above discussion of the sources of error in colorimetry that accurate and precise measurements by this method would be hard, if not impossible, to obtain. **Fortunately, however, most of these errors can be avoided, or reduced to a satisfactory minimum, by carefully worked-out procedures and good technique, together with the use of a good colorimeter or carefully matched color comparison tubes.** A number of colorimeters possessing optical and mechanical accuracy are described in the literature (see Bibliography) and some of the more commonly used types are described in detail in Chapter II.



In conclusion, the author wishes to emphasize the importance of studying the various sources of error as they affect each colorimetric method employed, and it is earnestly hoped that workers in this field will coöperate in improving (and "weeding out" if necessary) some of the less accurate and imperfectly studied methods given in this treatise. It is mainly with this idea in mind that these procedures have been included. As far as possible, the author has endeavored to state the limits of accuracy of each method, but, unfortunately, such data are not available at present for a number of the procedures; in others the data are given only for very limited applications.

## CHAPTER VI

### COLLOIDS AND COLORIMETRIC STABILIZERS

IN most colorimetric methods of analysis we deal with true solutions whose colors are due either to the presence of colored molecules or to colored ions. That is to say, the component substances of the solutions are molecularly (or ionically) dispersed in each other. Some methods, however, are based upon the formation of colored colloid suspensions. These suspensions, or so-called colloidal solutions, are in some cases fairly stable and may be compared in a colorimeter in the usual way without the introduction of a serious error due to aggregation of the particles. On the other hand, some of the suspensions are so unstable that the color matching must be made very rapidly and great care must be taken to prepare the "unknown" and "known" simultaneously in order to secure approximately the same aging effects.

**Protective Colloids and Other "Stabilizers."**—In the case of finely divided suspensions it is possible to increase their stability, i.e., prevent or at least decrease their rate of coagulation and precipitation, by adding a protective colloid or stabilizer such as gelatin, gum arabic, gum acacia, starch, egg albumin, etc.

It was observed as early as Faraday<sup>1</sup> (1857) that the addition of a small amount of "jelly" (probably gelatin) prevented the coagulation and precipitation of metal sols. Such organic substances as gum arabic, gelatin, and the proteins are themselves largely colloidal in solution and hence they have been termed "protective colloids." It is held that they form a film around each suspended particle in a sol and thus protect them from the action of substances that ordinarily would cause coagulation. For example, arsenious sulfide sol containing a little gelatin is not coagulated by the addition of electrolytes in amounts much greater than would cause precipitation if no protective colloid were present. This protective action is known as the

<sup>1</sup> Michael Faraday, *Phil. Trans.*, **147**, 184 (1857).

*envelope theory of protection* and was enunciated by Bechhold.<sup>2</sup> The mechanism of such action has been quite definitely demonstrated by Jacques Loeb<sup>3</sup> by a comparison of the stability of protein solution with that of dispersions of protein-coated collodion particles.

The protective power of these hydrophilic or "protective" colloids varies greatly and is measured in terms of the "gold number," a term due to Zsigmondy.<sup>4</sup> The "gold number" is the maximum number of milligrams of protective colloid that may be added to 10 cc. of a standard gold sol without preventing a change of color from deep red to violet shades by 1 cc. of a 10 per cent sodium chloride solution. It must be remembered, however, that the "gold numbers" are useful solely as very rough indices of relative protective powers, because the "gold number" of a given "protective colloid" depends so largely upon many conditions. Lack of space prohibits discussing here further aspects of this subject.

In addition to stabilizers of the above type, there are those of the so-called "solution link" type, e.g.,  $\text{H}_2\text{S}$ ,  $\text{FeCl}_3$ , etc., described by Thomas and Frieden.<sup>5</sup> Examples of colorimetric methods involving such stabilizing action are: the sulfide methods for lead, copper, iron, and antimony in which  $\text{H}_2\text{S}$  is the stabilizer. For a theory of the mechanism of such stabilizing action see the paragraphs on "The Solution Theory of Colloid Stability," page 95.

Some examples of the use of hydrophilic or "protective" colloids in colorimetric analysis are: (1) gum arabic in the determination of antimony as the sulfide, (2) gum arabic in the determination of bismuth as the iodide, (3) gum arabic in the determination of selenium, (4) gelatin in the determination of lead as the sulfide, (5) gelatin in the determination of acetylene by ammoniacal cuprous chloride, and (6) gum acacia in the determination of phenols in blood.

Yoe and Hill<sup>6</sup> have recently shown that the addition of a small amount of starch solution to a solution containing aluminum gives a stable suspension of the deep red aluminum lake produced by the addition of the dye aurin tricarboxylic acid. The use of starch solution permits determining colorimetrically aluminum in much higher

<sup>2</sup> Z. physik. Chem., **48**, 385 (1904).

<sup>3</sup> J. Gen. Physiol. **5**, 479 (1923).

<sup>4</sup> Z. anal. Chem., **40**, 697 (1901).

<sup>5</sup> A. W. Thomas and A. Frieden, J. Am. Chem. Soc., **45**, 2522 (1923); Thomas, J. Chem. Education, **2**, 323 (1925).

<sup>6</sup> See p. 109; also Yoe and Hill, J. Am. Chem. Soc., **49**, 2395 (1927).

concentrations than would be possible without the presence of a protective colloid to stabilize the colored suspension. Moreover, the presence of this protective colloid does not impair the sensitivity of the determination. This latter statement must not be taken to imply that such would *always* be the case with other constituents or with other protective colloids. In each case precaution must be taken to determine what effect, if any, the presence of a given protective colloid has.

The effect of protectors on the color changes of benzopurpurin when its solutions are acidified has been studied by Jerome Alexander.<sup>7</sup> He observed that "in the case of a pure dilute solution of benzopurpurin, the addition of dilute mineral acids quickly changed the color from bright red to dark blue, reminding one of the change of pure colloidal gold. Stronger acid coagulated the dye, which settled out of solution but which could be redissolved with restoration of the original color, by neutralizing the acid with alkali. The same solution of benzopurpurin, to which gelatin or gum arabic had been added, gave with dilute mineral acids a claret-red solution. More concentrated acid changed the shade to chocolate-brown, without, however, causing any precipitate." (See also the following paragraphs on the effect of emulsoids on colored solutions.)

**Effect of Emulsoids on Colored Solutions.**—"The color of the solution of a colored substance of small molecular weight is little or not at all affected by the presence of an emulsoid which does not react chemically with the colored substance and which does not change the hydrogen-ion concentration of the solution. When the colored substance has a large molecular weight, the presence of an emulsoid may influence the color of the solution in a number of ways. Should the colored solution be a suspensoid, the emulsoid will protect it from coagulation, as for example, gelatin protects colloidal gold solution. Should an insoluble colored substance be liberated from its soluble form the emulsoid will prevent its precipitation, as is the case when an acid solution of Nile blue is made alkaline in the presence of egg albumin. Finally, if the colored substance happens to fade on standing, the emulsoid may retard the rate of fading. This is true of methyl violet and gum arabic in alkaline solution. In all such cases the emulsoid exerts its influence by retarding or preventing some change which would take place in its absence, and consequently the color of

<sup>7</sup> J. Soc. Chem. Ind., **30**, 517 (1911); also Seventh International Congress of Applied Chemistry.

the solution containing the emulsoid will differ from that of the same solution containing no emulsoid."<sup>8</sup>

In addition to the above influence of emulsoids on the color of solutions, there is one which depends on an equilibrium between the emulsoid and the colored substance. In connection with the colorimetric determination of hemoglobin as alkaline hematin, H. Wu and D. Y. Wu attempted to use as the standard of comparison pure alkaline hematin solution prepared by dissolving crystalline hematin in alkali. They found, however, that the color of the pure hematin solution was much weaker than that of the solution prepared from the corresponding amount of hemoglobin. The only difference between the two solutions was that the one prepared from hemoglobin contained protein, globin, while the other did not. Evidently the globin increased the color of the alkaline hematin. Serum, egg white, gelatin and other proteins added to pure hematin solution gave similar results, though differing quantitatively. Other emulsoids such as gum arabic, starch, agar agar, etc., were tried and all of them were found to influence the color intensity of the alkaline hematin. Another colored substance, Congo red, gave similar results.

"The influence of emulsoids on the color of alkaline hematin and Congo red solutions cannot be ascribed to a change in the alkalinity, for such a change of alkalinity as might be caused by the addition of the emulsoid was shown to have no appreciable effect in itself on the color of the solution. Moreover, the color of alkaline hematin and Congo red produced on the addition of an emulsoid cannot be obtained in its absence by mere change of reaction. Hematin and Congo red are acids and they should not combine with protein, which in alkaline solution also behaves as an acid. Chemical combination of hematin and Congo red with the polysaccharides is even more unlikely. That emulsoids of entirely different nature, such as egg albumin and gum arabic are, should exert a similar influence on the same colored substances shows that the influence is physical and not chemical."<sup>9</sup>

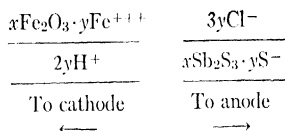
Wu and Wu point out that there are two ways in which a colored solution may be influenced physically by an emulsoid, namely, (1) by adsorption of the colored substance by the emulsoid, and (2) by adsorption of the emulsoid by the colored substance. In the former

<sup>8</sup> H. Wu and D. Y. Wu in J. Alexander's "Colloid Chemistry," Vol. I, p. 380. The Chemical Catalog Co., New York, 1926.

<sup>9</sup> H. Wu and D. Y. Wu, *loc. cit.*

case a layer of the colored substance is formed around each particle of the emulsoid, while in the latter case the reverse is true. The color of the "adsorption complex" in either case may differ in intensity and in shade from that of the particle of the free colored substance. The so-called "protein error" has long been recognized in the colorimetric determination of hydrogen-ion. From the work of Wu and Wu it is evident that the presence of emulsoids is a factor to be considered in colorimetry in general.

**The Solution Theory of Colloid Stability.**—"The origin of the solution forces and the reason for the electrical migration of colloidal dispersions of apparently insoluble substances such as ferric oxide, antimony sulfide, gold, etc., have gradually become apparent through years of research. It is now definitely known that these insoluble substances do not exist as such in colloidal solution. The particles in colloidal ferric oxide solution consist of a complex of  $\text{Fe}_2\text{O}_3$  and a soluble iron salt such as  $\text{FeCl}_3$ ; antimony sulfide hydrosol consists of a combination between  $\text{Sb}_2\text{S}_3$  and  $\text{H}_2\text{S}$ . Formulas may be roughly written from them (ignoring the hydrate water) as follows:  $x\text{Fe}_2\text{O}_3 \cdot y\text{FeCl}_3$ ;  $x\text{Sb}_2\text{S}_3 \cdot y\text{H}_2\text{S}$  where  $x$  and  $y$  are variable and  $x$  is always greater than  $y$ .<sup>10</sup> When an electric current is passed through these solutions, a brown precipitate of  $\text{Fe}_2\text{O}_3$  settles out at the cathode and chlorine is evolved at the anode in the iron oxide hydrosol, while in the antimony sulfide sol, a red deposit of  $\text{Sb}_2\text{S}_3$  is deposited at the anode and hydrogen gas is liberated at the cathode. This shows that the migrating ions are:



The ionization is not complete; it is in fact slight and the nature of the migrating ions is not quite so simple as indicated.<sup>11</sup> But it is not possible to make fine distinctions in a short discussion.

<sup>10</sup> These formulas give too simple a picture and are liable to mislead. For instance, the iron oxide sol written as  $x\text{Fe}_2\text{O}_3 \cdot y\text{FeCl}_3$  may give the idea that all the  $\text{FeCl}_3$  is free to ionize in solution or that just the  $\text{Fe}^{+++}$  is adsorbed. For academic purposes a better simplified picture is  $(x\text{Fe}_2\text{O}_3, z\text{H}_2\text{O}, y\text{FeCl}_3, z\text{Fe}^{+++})3y\text{Cl}_3^-$  where  $y$  is smaller than  $x$ , and  $z$  is very small in comparison with  $y$ . Private communication from Dr. Arthur W. Thomas.

<sup>11</sup> See J. Duclaux, J. chim. phys., 7, 405 (1909).

"The gradual realization of the fact that the 'impurities,' e.g., the  $\text{FeCl}_3$  or the  $\text{H}_2\text{S}$ , were essential parts of certain hydrosol particles gave rise to the so-called *Complex Theory*, a very simple statement of the complex nature of certain colloids.

"While the Complex Theory was accepted for many colloidal dispersions, hydrosols of noble metals such as gold and platinum were thought to be exceptions since it was believed that they could be prepared by electrically arcing these metals under pure water. This was disproved by Beans and Eastlack,<sup>12</sup> who demonstrated that colloidal platinum could be formed in pure water due to the fact that platinum oxidizes in the arc thus generating an electrolyte which became part of the dispersed phase. Gold was shown to require the presence of minute amounts of certain salts, in fact those which form stable chemical compounds of gold.

"It is therefore not difficult to see the origin of the solubility forces, since insoluble substances in colloidal solution are actually a part of a complex aggregate containing a soluble component. Evidence for solution forces as the reason, or at least one of the reasons for colloid solution stability has been given by Thomas and Frieden.<sup>13</sup> A simple experiment may be cited. Addition of alcohol followed by ether to a hydrosol of  $x\text{Fe}_2\text{O}_3 \cdot y\text{FeCl}_3$  did not affect it. Alcohol promptly precipitated a hydrosol of  $x\text{Fe}_2\text{O}_3 \cdot y\text{Fe}_2(\text{SO}_4)_3$ . Ferric sulfate is insoluble in alcohol." (Thomas.)

**Interference Eliminators.**—Another class of agents employed in colorimetric analysis may be properly termed "interference eliminators." For example, in the determination of ammonia by Nesslerization, calcium and magnesium are precipitated by Nessler's reagent and, hence, would interfere with the analysis. They may be removed by precipitating in the usual way and filtering, but it is more convenient to add Rochelle salt which holds them in solution. Another example of the use of an interference eliminator is in the determination of phosphorus as phosphomolybdate. Here, 2 or 3 drops of ammonium oxalate are added before adding the phosphate reagent in order to prevent the calcium precipitating as calcium phosphate.

**Nesslerization.**—The yellow to brown color produced by the reaction of ammonia with Nessler's reagent is fairly stable and (when the ammonia concentration is not too high) gives a solution which appears

<sup>12</sup> Beans and Eastlack, J. Am. Chem. Soc., **37**, 2667 (1915).

<sup>13</sup> A. W. Thomas and A. Frieden, J. Am. Chem. Soc., **45**, 2522 (1923).

water-clear and homogeneous to the naked eye. However, Robertson and Hisey<sup>14</sup> have recently made an extensive experimental study on the nature of the Nessler color and conclude that it is due to colloiddally dispersed particles in suspension and not in true solution.

**Peptization.**—Finely divided particles<sup>15</sup> may be obtained either by condensation from vapor or solution (precipitation) or by disintegration of larger masses. The disintegration is frequently called *peptization*, especially if it is done chemically as opposed to mechanical or electrical disintegration. The word *peptization* is due to Thomas Graham.

In a study of the reduction of certain vat dyes by alkaline sodium hyposulfite, Yoe and Edgar<sup>16</sup> employed a colorimetric method of measuring the amount of dye reduced. Their results indicated that the reaction between the dye and the alkaline hyposulfite is very rapid, that an insoluble crystalline reduced dye (dark blue) is first formed, and that the latter is then *peptized by hydroxyl ions to form a dark blue sol*. The dark blue sol was matched in a colorimeter against a standard sol similarly prepared or against a standardized blue glass. The *rate* of peptization, and the *amount* of *dye stuff* peptized by a given solution, depend upon the state of subdivision of the dye. The rate of reduction is much faster than the rate of peptization.

**Color and Degree of Dispersion.**—It has been shown by experiment that the color intensity of substances varies with the degree of dispersion and that it attains a *maximum in the realm of colloid dispersion*. It is well known that colloidal solutions of certain salts, e.g., the sulfides of lead, copper, arsenic, etc., show such a marked color, even in very low concentrations, that this property may be used for their recognition. The intensity of color of these colloidal solutions may at times exceed that of the aniline dyes. For example, if the color intensity of fuchsine be represented by an arbitrary value of 5, that of colloidal hydrous ferric oxide is about the same, while that of arsenious trisulfide is 100, and that of colloidal gold about 2000 (The Svedberg).<sup>17</sup> This variation in color intensity is strikingly illustrated in the case of colloid gold. (See Fig. 41.) The great variation

<sup>14</sup> Private communication from Dr. J. H. Robertson.

<sup>15</sup> Cf. W. D. Bancroft, *Applied Colloid Chemistry*, p. 162. McGraw-Hill Book Co., New York, 1921.

<sup>16</sup> Yoe and Edgar, *J. Phys. Chem.*, **27**, 65 (1923); Yoe, *ibid.*, **28**, 1211 (1924).

<sup>17</sup> Cf. Ostwald-Fischer, *Theoretical and Applied Colloid Chemistry*, 2d ed., p. 63. John Wiley & Sons, Inc., New York, 1922.



in color phenomena exhibited by colloid metals was observed by Michael Faraday three-quarters of a century ago, and he pointed out that the degree of dispersion is largely responsible for color formation.

This color variation with the size of the particles is also exhibited by certain dyes, as seen in the color changes in Congo-rubin sols. "The particles of this dye sol have diameters between those of colloids and molecular dispersoids. It may be suddenly transformed to a blue-violet or blue solution not only upon addition of acid but also

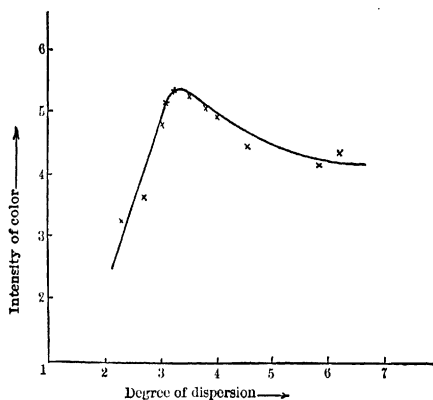


FIG. 41.—Relation of color intensity of colloid gold to its degree of dispersion according to The Svedberg.

(By permission from Ostwald-Fischer, "Theoretical and Applied Colloid Chemistry," 2 ed. p. 63, John Wiley and Sons, Inc., New York, 1922).

benzopurpurin. (See page 93.) Ultramicroscopic observations by Alexander<sup>19</sup> showed that aggregation of the colloiddally dispersed dye is accompanied by a change in color. This is no doubt true in the case of many other dyes.

Not only does the intensity of color vary with the degree of dispersion but also the beauty and *variety*. "The *order* in which the colors change from one to the other as the degree of dispersion changes seems also to be definite. As a rule, the most highly dispersed colloid metals are yellow or orange; in other words, they absorb violet and blue light. As the degree of dispersion decreases, the color passes from yellow

by the addition of any neutral salt or even alkaline substances. The dye behaves like a red gold sol in many respects and it may be used as a gold sol substitute. The color transition of Congo-rubin is reversible by dilution, by raising the temperature, by addition of alcohol, etc."<sup>18</sup> Another

illustration of color change is the dye

<sup>18</sup> W. Ostwald, *et al.*, Practical Colloid Chemistry (Translation of 4th ed.), p. 70. E. P. Dutton and Co., New York, 1927.

<sup>19</sup> J. Soc. Chem. Ind., **30**, 517 (1911).

through orange to red, violet, blue, and finally green. The absorption maximum gradually moves towards the side of the greater wavelengths as the degree of dispersion decreases."<sup>20</sup>

As mentioned above, the great intensity of color shown by colloidal dispersed metals may at times even exceed that of the aniline dyes. It is natural that this property of the metals (especially the so-called noble metals) should have been called upon for analytical purposes and it is not surprising that one of the oldest and best-known methods for demonstrating the presence of traces of gold consists in reducing the gold to the colloid condition. The *purple of Cassius* test for gold is a typical illustration of the production of gold in the colloid state of division and its subsequent precipitation in the form of an "adsorption compound" through a second colloid. The first step in this test is accomplished by reducing the gold salt with stannous chloride. In this way colloid gold and colloid stannous acid are formed, and these in turn unite to form the well-known, reddish-violet colored suspension or precipitate.

**Concentration by Adsorption on a Crystalline Solid.**—Another application of colloid chemistry to colorimetry is made in the case of the determination of zinc by the potassium ferrocyanide method. This is a turbidimetric method described by Breyer<sup>21</sup> in which zinc is precipitated as a colloidal suspension by potassium ferrocyanide solution. Meldrum has applied this method to the determination of zinc in water, and Birckner<sup>22</sup> to the determination of zinc in various food products. Bodansky<sup>23</sup> modified the Breyer-Birckner method and used it to determine the zinc content of marine organisms. Solid calcium citrate is employed in the recovery, i.e., concentration, of the colloidal zinc sulfide. A better recovery is obtained when the calcium citrate is formed in the solution than when it is added pre-formed. It is thought that the calcium citrate adsorbs the colloidal zinc sulfide particles and hence effects a better recovery. This is in line with Bancroft's<sup>24</sup> observation: "There is some evidence to show that when a

<sup>20</sup> Ostwald-Fischer, *loc. cit.*, pp. 65-66. For further details regarding this relation between color and degree of dispersion, see Wo. Ostwald, *Kolloidchem. Beih.*, **2**, 409 (1911), and *Licht und Farbe in Kolloiden*, Th. Steinkopff, Dresden and Leipzig, 1924.

<sup>21</sup> W. W. Scott, *Standard Methods of Chemical Analysis*, 4th ed., p. 607. D. Van Nostrand Co., New York, 1925.

<sup>22</sup> *J. Biol. Chem.*, **33**, 191 (1919).

<sup>23</sup> *J. Biol. Chem.*, **44**, 399 (1920); *J. Ind. Eng. Chem.*, **13**, 696 (1921).

<sup>24</sup> *J. Ind. Eng. Chem.*, **13**, 153 (1921).

colloidal solution is precipitated<sup>25</sup> the finer particles attach themselves to the coarser ones." The zinc sulfide is finally dissolved and reprecipitated as a finely divided suspension by the addition of potassium ferrocyanide.

**Concentration by Co-Precipitation with a "Collector."**—In the colorimetric method (thiocyanate method) of Stokes and Cain<sup>26</sup> for the determination of iron, by far the greater number of cases require concentrating the iron by precipitation. An almost indefinitely small quantity of iron may thus be determined in an indefinitely large amount of material, the only limit being the solubility of the iron precipitate in the solution. It is obviously impossible to collect, on a filter, traces, say a thousandth of a milligram of ferric hydroxide or sulfide distributed in a finely divided state through a considerable volume of an otherwise clear liquid. Stokes and Cain, therefore, employ the method which has been occasionally used successfully in other cases, of mechanically carrying down the minute amount of finely divided precipitate by a relatively large amount of another precipitate, which, when practicable, is generated simultaneously with the iron precipitate. We may designate this secondary precipitate as the "collector." Various substances suggest themselves as collectors; their number is limited by the following considerations. A collector must be sufficiently insoluble, so that but a small amount of a possible impure foreign substance need be introduced; it must be of such physical consistency as to enable it to carry down all suspended precipitates and must therefore be amorphous and flocculent, not granular or crystalline; it should not be gelatinous or otherwise difficult to wash out in the filter, neither should it be of such consistency as to run through the filter on washing; it must be easily soluble in 7 per cent thiocyanic acid and must neither interfere with the ferric thiocyanate reaction nor in the presence of mercuric thiocyanate impart a color to amyl alcohol; or, if it does not meet these requirements, it must be capable of easy separation from the iron. Aluminum hydroxide would be the ideal collector were it not for the fact that it dissolves slowly and imperfectly in thiocyanic acid, and thus frequently prevents complete solution of the accompanying ferric hydroxide. Repeated experiments by Stokes and Cain showed that it

<sup>25</sup> E. F. Burton, *The Physical Properties of Colloidal Solutions*, 2d ed., p. 173. Longmans, Green and Co., New York, 1921.

<sup>26</sup> *J. Am. Chem. Soc.*, **29**, 409 (1907).

is not to be depended on, and they have, therefore, employed it only in special cases where it was removed before final treatment of the precipitate with thiocyanic acid. The iron is precipitated either as sulfide or as ferric hydroxide. The hydroxide precipitation is employed in the absence of materials which have a solvent action such as citrates, tartrates, sugar and many other organic substances, pyrophosphates, arsenites, arsenates, antimonates, etc. The usual collector for ferric hydroxide is hydrated manganese peroxide. The sulfide precipitation is used when from the presence of any of the just-mentioned substances, hydroxide would remain in solution. It is also used when other sulfides insoluble in ammonium or sodium sulfide are practically absent. The best collector for iron sulfide is cadmium sulfide. In this case the cadmium sulfide is redissolved and the iron reprecipitated as hydroxide with manganese dioxide as collector. In many cases the choice between the methods is optional. When there is reason to fear the presence of traces of organic matter, as in the case of materials which have been treated in wooden vessels in the process of manufacture, or when arsenic or other prejudicial substances may be present, as in the cruder reagents, the sulfide method is more accurate. For example, pure sodium chloride gave Stokes and Cain identical results by either method, while a sample of the best commercial chloride gave decidedly too low results with the hydroxide method.

Special care is necessary in sampling the substance, and, wherever practicable, duplicate determinations should be made on portions of the same solution, as it frequently happens that different samples, especially of crystallized substances, taken from the same bottle show widely varying results, owing to the irregular distribution of the iron.

Another illustration of the use of a "collector" is in the colorimetric determination of tungsten by the method of Travers.<sup>27</sup> This method is based upon the reduction of tungstic acid by titanium chloride, giving a blue colored oxide of tungsten that remains in colloidal suspension under certain conditions. If the sample is an alloy, it is treated directly with aqua regia; if a mineral, it is fused with sodium sulfite and the mass taken up with aqua regia. Most of the tungsten is precipitated as tungstic acid (along with the silica) but the separation is not quantitative because of the presence of metatungstic acid. The filtrate, which should contain iron (10 per cent is sufficient), is treated with ammonium hydroxide until it is just alkaline to litmus.

<sup>27</sup> Compt. rend., **166**, 416 (1918); cf. *ibid.*, **165**, 408 (1917).

The ferric hydroxide thus precipitated entrains the tungstic acid and hence serves as a "collector." The precipitate is then washed free from sodium salts, dissolved on the filter in 6 N hydrochloric acid, and the solution added to the major part of the tungstic acid solution, after the latter has been freed from silica by any of the usual methods and taken up in hydrochloric acid. The combined solutions are evaporated to a volume of about 2 cc., cooled, diluted to 40 cc., 5 cc. of titanium chloride solution added, and the resulting blue colloidal solution diluted to 50 cc., mixed, and compared at once with a standard similarly prepared.

**Factors That May Influence the Size of Colloid Particles.**—In addition to the effect which stabilizers have on the size of colloid particles there are such factors as the hydrogen-ion concentration, concentration of electrolytes, temperature, light, nature of dispersion means, etc. Any one or more of these may seriously influence the degree of dispersion of colloid particles and hence the accuracy of the determination. It is, therefore, necessary to carry out all procedures (which deal with colloid suspensions) under carefully studied and well-defined conditions in order to attain the highest degree of precision and accuracy. Perhaps the most important precaution to take is to carry out the preparation of the unknown and standard under as nearly identical conditions as possible.

## CHAPTER VII

### GENERAL DIRECTIONS FOR USING A PRECISION COLORIMETER

BY PHILIP ADOLPH KOBER, DIRECTOR OF RESEARCH, G. D. SEARLE  
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ALTHOUGH almost anyone can use a gravimetric balance for rough weighing, yet the correct use of a precision balance for accurate weighing is not accomplished without considerable study and practice as well as the standardization of both balance and weights. Likewise, rough matching of colors or light can be practiced by the uninitiated, but the precise use of a photometric balance, a colorimeter or nephelometer, requires the consideration of an almost equal number of factors, of which the following are the most important:

**The Choice of Instrument.**—In order to eliminate the errors arising from a meniscus, highest precision in colorimetry can only be practiced with a single or double plunger type of instrument. This type of instrument is the only one thus far developed that measures the light or color between two parallel planes of glass, thus giving a very sharp differentiation to the heights of solution contained in the cells or cups. The plunger, with its mechanical and accurately adjustable stage, allows the measurement of the height to be made to any desired accuracy. In practice this accuracy has been limited to a tenth of a millimeter, which subdivision is usually obtained by means of a vernier. If the accuracy of observation warranted it, this reading of the scale could, of course, be made to a hundredth of a millimeter or less if necessary.

**Specifications of a Plunger Type of Instrument.**—(a) *Good Optical Construction.*—One of the first specifications of a precision colorimeter is good optical construction. The parts must be made from flawless optical glass of minimum tint or having little or no absorption of light. Plungers made hollow with fused bottoms having parallel planes show much less absorption of light than do the best of

solid plungers. The optical parts should be so mounted and incased as to eliminate stray reflections, and as much as possible to prevent deposition of dust and condensation of moisture. Even the best of instruments require from time to time a thorough cleaning of all of the optical parts, which involves the complete taking apart of the eyepiece and prism house. The optical arrangement should be so constructed that, if necessary, readjustments or refocusing of the parts can be made. In addition to the glass parts of the optical system, suitable apertures and diaphragms are required. As a rule those instruments having small diaphragms are more sensitive than those with large diaphragms, but both extremes are to be avoided. Optical systems having little or no balsam in their construction are to be preferred to those having large surfaces balsamed. For a discussion of the reflector of a colorimeter, see below under Mechanical Construction.

(b) *Mechanical Construction*.—The accuracy obtainable with an instrument of good optical construction is often lost by the operation of a poor mechanical arrangement. Since the colorimeter, if it is sensitive, is like a precision gravimetric balance, the equality between the two sides is seldom maintained for a long time. It would not be a sensitive colorimeter if it did not respond to variations in temperature, dust, and moisture depositions. For this reason a good colorimeter should have its mechanical parts adjustable so that, if necessary, the instrument can be readjusted from time to time, and a new balance of its sides obtained. Instruments having a single reflector must be moved in relation to the light source so that equal illumination is obtained in both sides of the instrument and fields. This is a sort of hit-and-miss adjustment. Much to be preferred are those instruments that have split or double reflectors, i.e., a separate reflector for each side, so that when the light in relation to the instrument is approximately in the right position, the final adjustment to equality can be made by means of a micrometer movement of the split reflectors with ease and precision. Adjustable scales or vernier are important for accurate and convenient use of a precision colorimeter. In the moving parts the great requirement is the absence of lost motion, so that the eye can be trained to observe the slightest differences in color or light. In instruments that have considerable lost motion one can never be sure of slight movements of cups or plungers and therefore one does not know whether differences in observation really represent differences in the heights of the solutions.

The turned knobs or milled heads operating the stages, i.e., the movement of the cups or plungers, should be so located that they are not close to those operating the reflectors, in order to avoid accidental displacement of the reflector during a comparison. Where many determinations are to be made, convenience in reading the scale is a factor, for any operation which increases the fatigue or inconvenience during the determination will have its effect upon the sensitivity of the observer's eyes. In a like manner, the instrument should be so constructed as to prevent glares and stray light from getting to the observer's eyes during the use of the instrument. In some of the precision instruments the eyepiece is placed in a horizontal position, enabling the observer to match the colors or light in a sitting position, while other instruments have the eyepiece in a vertical position, which, unless the instrument is placed on a low stand or table, requires a standing position on the part of the observer. The instruments with a horizontal eyepiece have the disadvantage that unless the light source is carefully and completely shaded, stray light will get to the observer's eyes and make matchings more difficult and less sensitive. With instruments having a vertical eyepiece, the use of a black table top will practically exclude stray light from the observer's eyes. In short, if the proper arrangements are made with both types of eyepiece, there is no choice between them so far as convenience and accuracy is concerned.

**Care of a Precision Instrument:** (a) *When in Use.*—The necessity of caring for a delicate machine or instrument ought to be apparent to most workers, but it might not be amiss to point out some of the major precautions in handling an instrument. The cups and the plungers should, of course, be kept perfectly clean, and rinsed once or twice with the solution to be used for determination. The cups must not be filled too high so that they overflow on to the reflectors, staining or corroding them. Care should also be taken that the plungers do not strike the bottom of the cups too hard while taking the so-called "zero-point" reading. Where long cups are used the zero-point must be measured by inserting into the cups a wooden or metal rod with parallel ends and of known length, usually called a "zero gauge." The zero-point should be taken with each individual cup and the cup always used on the same side and in the same position. By making a small mark on the top of the cup this precaution can be easily carried out. This standardization of the instrument in relation to the cups is



necessary, as it is seldom that the thickness of the glass used in the cups and the thickness of the supports are exactly alike. It is, of course, obvious that the bottoms of the plungers and the cups should be kept free from scratches and cracks.

(b) *When Not in Use.*—The instrument should be kept covered to keep out the dust, but it is also important that the room containing the instrument be kept at a uniform room temperature and away from excessive moisture and fumes, particularly that of ammonia.

**Source of Light.**—The source of light under proper conditions can be either daylight or artificial light. The advantage of daylight is, of course, that it enables one to measure almost any tint without the use of light filters, but it has the disadvantage that it cannot be controlled or is seldom constant in intensity. Since temperature variations are very great near a window, and since it is difficult always to have a northern exposure, the use of daylight is very difficult for uniform and accurate work. By using artificial light, and, if necessary, employing suitable light filters, close approximation to daylight can be obtained, as well as light of special composition for certain particular purposes.

**Precautions to be Taken Relative to the Observer.**—The accuracy of photometric work greatly depends also upon the care taken with the observer's eyes. Although there are a number of persons whose sensitivity to colors is very low and who are therefore incapable of making any accurate colorimetric determinations, most workers do not realize that the sensitivity of the eyes can be greatly increased by judicious use and training. The human eye is like some electromotive cells, in that it is easily polarized, i.e., its greatest sensitivity or accuracy does not hold for a very great length of time, without many periods of rest. Therefore the period of observation should be as short as is conveniently possible. A good practice is to make a rough adjustment with one eye and the final setting with the other. A number of photometric observers have found it an aid to massage the eyes with hot or cold water several times a day, and it is a fact that the organ of sight, like other organs and tissues, can be strengthened and its value and accuracy greatly increased by proper use.

The best environment for photometric observation is that of a darkened but well-ventilated room whose walls and contents have a dark tint. It, of course, follows that the light source must be well screened so that at no time the observer's eyes are partially blinded by the glare.

For beginners, it is very important that a reasonable time be allowed for practice determinations, so that they get accustomed to the instrument and the environment of the instrument. Occasionally an observer is more sensitive to certain colors or tints than to others and by suitable choice of light filters this sensitivity can be easily utilized. Quietness and freedom from extraneous disturbances are also essential to accurate photometric work.

**Method of Obtaining a Photometric Balance.**—The light source must be adjusted with relation to the instrument, or the instrument must be adjusted to the light source so that equal illumination on both sides of the instrument is produced. Once this is obtained it is best either to fasten the instrument and light source in position, or, if that is not possible, to mark on the table or support the exact position of each. If the instrument has split reflectors, equal illumination of the fields is finally obtained, after the cups, previously marked, are placed in position with distilled water or the solvent used in the actual determination, with the plungers immersed in the liquid, and the separate reflectors adjusted until equal illumination is obtained. Either before this or afterwards, the empty cups are placed in the instrument in the same position and the instrument operated gently until the plunger touches the bottom of the cups, if the cups are the usual short ones. (For directions with long cups, see page 105.) In this position there is zero height of liquid between the cups and the plungers, and the scale read with the vernier should read exactly zero. If this is not the case, the scale or the vernier must be adjusted until the reading is exactly zero.

Now, if everything is in proper adjustment, some standard solution is put into the cups, after carefully rinsing with the same solution, and one side, say the right side, is set at 20.0 or 30.0 mm. exactly. The observer now tries to obtain equal color or tint by operating the other side. A series of five or ten settings should give a good average of the reading on the right side. If they do not, it may be due to some poor adjustment of instrument or light source or to an idiosyncrasy on the part of the observer. In the former case, a readjustment will correct this inequality, or in both cases it may be taken care of as follows:

The plunger on the left side is set exactly on the average obtained above, and the standard solution on the right side is discarded in favor of the next solution, say one-half or one-third standard, and after

making a series of settings using only the right side, the average is compared or used in calculation with 20.0 or 30.0 the original reading on the right side. In other words, the setting on the left side does not come into consideration, the solution there acting only as a tare. If the solution obeys Beer's law the reading for one-half standard should read exactly twice that of the standard, and that of one-third standard should read exactly thrice that of the standard. This check on Beer's law should be made by those using a colorimetric reaction for the first time, as it enables the observer to check not only the instrument and his observations, but also the sources of error inherent in the reaction or the method of producing the reaction. If the solution does not obey Beer's law, a colorimetric curve must be constructed. (See Chapter IV.)

In actual routine analyses it is best to make the balance first with the unknown solution and then to substitute the standard solution on the right side and vary the height of the standard until a setting is obtained, as it makes the calculation very much simpler, as is shown on pages 55 and 70.

The number of readings necessary for accurate work varies greatly with the type of solution of substance to be determined. Other things being equal, the greater the number of readings the nearer accurate is the result. Where many determinations are to be made, many readings for each solution make for greater eye fatigue. Also, some solutions change with time, i.e., they are not constant for a long period of time. Moreover, the experience and the skill of the observer must be taken into account. As a rule, many readings are taken only when isolated analyses are to be made and the analyst has had no opportunity to keep in photometric practice. A trained and experienced observer usually varies the number of readings in accordance with their agreement. If three or four readings agree closely or check, further readings would hardly be of any value for most analytical purposes.

## PART II

### INORGANIC

## CHAPTER VIII

### ALUMINUM

#### DETERMINATION OF ALUMINUM BY "ALUMINON" (THE AMMONIUM SALT OF AURIN TRICARBOXYLIC ACID)

##### METHOD OF YOE AND HILL

This method is based upon the use of the ammonium salt of aurin tricarboxylic acid, the new reagent for the detection of aluminum recently described by Hammett and Sottery.<sup>1</sup> This dye forms a bright red lake with aluminum in acetic acid acetate solution and the lake is fairly stable in ammonium hydroxide carbonate solution.

The method is applicable to the determination of small quantities of aluminum in salts, minerals, and water. A very faint pink coloration is developed when one part of aluminum is present in 25 million parts of solution, or 0.002 mg. in 50 cc. The results of an extensive experimental study of the method are briefly summarized in the Notes on pages 111-114.<sup>2</sup>

##### Reagents.

1. Hydrochloric acid, 4 N.
2. Hydrochloric acid, 1 N.
3. Nitric acid, 1 N.
4. Ammonium hydroxide, 5 N.
5. Sodium hydroxide, 6 N.
6. Ammonium acetate, 3 N.
7. Ammonium carbonate, 5 N. (See Note 6.)
8. Methyl red, 0.05 gram in 100 cc. 95 per cent alcohol.

<sup>1</sup> J. Am. Chem. Soc., **47**, 142 (1925).

<sup>2</sup> For a more detailed report see: J. H. Yoe and W. L. Hill, J. Am. Chem. Soc., **49**, 2395 (1927).

9. "Aluminon," 0.1 per cent solution. "Aluminon" (the ammonium salt of aurin tricarboxylic acid) may be prepared by the second method of Ger. pat. 49, 970 (1889), Friedländer II, 50; or it may be obtained from the Fales Chemical Co., 74 Cortlandt St., New York City.

10. Standard aluminum chloride solution. Dissolve 8.952 grams of pure aluminum chloride,  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ , in distilled water, dilute to a liter, and thoroughly mix. (See Note 1.) One cubic centimeter of this solution contains 1 mg. of aluminum. Dilute 10 cc. of this stock solution to a liter, and mix thoroughly. The diluted solution contains 0.01 mg. of aluminum per cubic centimeter. If desirable, the stock solution may be checked gravimetrically by the method of Blum.<sup>3</sup>

**Procedure.**—If the substance is a solid, dissolve in 15 cc. of water an amount sufficient to have an aluminum content between 0.005 and 0.5 mg., preferably below 0.1 mg. (see Note 3); if a liquid, adjust the concentration by dilution or evaporation to give a solution containing between 0.005 and 0.5 mg. (preferably below 0.1 mg.) of aluminum per 15 cc. In case an acid is required to dissolve the solid sample, or the liquid sample is acid, the hydrogen-ion concentration of the sample solution must be adjusted to a *pH* value of about 6.5. A solution which shows a *faintly* acid reaction to neutral litmus paper is satisfactory. Transfer the sample solution to a Nessler tube, add 5 cc. of 1 N hydrochloric acid, and 5 cc. of 3 N ammonium acetate solution. Dilute to 30 cc., add 5 cc. of the "Aluminon" reagent, mix, and let stand 5 minutes to allow the lake to form. (See Note 8.) Now add slowly with constant stirring 5 cc. of 5 N ammonium hydroxide and 10 cc. of 5 N ammonium carbonate solution, dilute to 50 cc., mix thoroughly, and after 25 minutes compare with standards prepared along with the unknown and similarly "aged." (See Note 6.) The comparison may be made in Nessler tubes or in a Kennicott-Campbell-Hurley colorimeter.

To determine aluminum in water, acidify 200–300 cc. of the sample with 4 N hydrochloric acid, evaporate in a platinum, silica, or porcelain dish to dryness on the water-bath, and ignite at dull redness for a few minutes to destroy any organic matter and to dehydrate the silicic acid. Treat the residue with 1 cc. of 4 N hydrochloric acid, warm, add 5–10 cc. of hot distilled water, digest a minute or two, filter,

<sup>3</sup> J. Am. Chem. Soc., **33**, 1282 (1916); also, in a little more detailed form as Sci. Paper No. 286, U. S. Bureau of Standards, 1916.

and wash with hot water. Dilute the filtrate to a volume of 25 cc., heat to boiling, add one drop of methyl red, and precipitate the aluminum and iron by the addition of ammonium hydroxide until the color change of the indicator is just reached. Digest on the hot-plate a few minutes and filter through a 7 cm. filter paper. If the iron content is small (see Note 5), dissolve the precipitate by pouring 5 cc. of warm 1 N hydrochloric acid through the filter several times, allowing the filtrate to run into a Nessler tube. Wash the filter several times with small volumes of hot water, adding the washings to the filtrate. Cool the filtrate to room temperature (see Note 7), add 5 cc. of 3 N ammonium acetate solution, and complete the procedure as directed in the first paragraph above.

When considerable iron is present, dissolve the precipitate of aluminum and iron hydroxides in 2-3 cc. of 5 N nitric acid, transfer the solution to a casserole, evaporate on the hot-plate to a volume of about 1 cc., make strongly alkaline with sodium hydroxide solution, heat to boiling, filter, and wash several times with hot water. Add hydrochloric acid to the filtrate until it is *just* acid to neutral litmus paper, transfer to a Nessler tube, cool to room temperature, and continue the analysis according to the procedure in the first paragraph above.

#### Notes.

1. Aluminum chloride hexahydrate in a high state of purity may be obtained from the anhydrous aluminum chloride of commerce.<sup>4</sup> The commercial salt is dissolved in water and filtered through glass wool. The solution is then mixed with concentrated hydrochloric acid, cooled in a freezing mixture, and saturated with dry HCl gas. Fine, white crystals of aluminum chloride hexahydrate separate. These are washed by decantation with concentrated hydrochloric acid until free from iron, sucked dry, and exposed to the air on porous tile until free from hydrogen chloride. The salt adsorbs water slowly from moist air, but at 20° C. it will dry to constant weight in 48 hours.

2. Since aluminum is one of the most common impurities in chemical reagents, great care must be taken that it is not present in any reagent employed in the analysis.

3. A very faint pink coloration is developed with one part of aluminum in twenty-five million parts of solution, or 0.002 mg. in 50 cc.

<sup>4</sup> L. M. Dennis, Z. anorg. Chem., 9, 39 (1895).

The upper limit with Nessler tubes, or with a colorimeter employing large volumes of solutions, is 0.5 mg. in 50 cc. on account of the intensity of color; although the lake does not coagulate until after several hours with quantities of aluminum up to 1 mg. in 50 cc., unless a considerable excess of ammonia is added. Starch may be used as a protective colloid, thus permitting the use of a higher aluminum content in the sample. In such cases, on account of the deeply colored solution, a colorimeter must be employed which permits using a small volume of sample. A Kleinmann micro-colorimeter or small Duboscq colorimeter should be satisfactory.

4. The following statements refer to quantities actually present in the Nessler tube at the time of lake formation. Phosphates prevent the formation of the lake; however, 2 mg. of  $\text{PO}_4$  ion is without noticeable effect. Silica in quantities greater than 1 mg. of  $\text{SiO}_2$  gives results which are appreciably low. As much as 1 mg. of chromium (either as chromate, or as chromic ion), cobalt, nickel, or manganese gives results which run from 6 to 15 per cent high, as also does magnesium in quantities greater than 1 mg. Calcium in quantities greater than 1 mg. produces a turbidity and consequently high results. Iodide, nitrate, and nitrite ions do not interfere, though sulfide and sulfite ions reduce the dye and thus destroy the color.

5. Iron interferes with the test, since it forms a violet lake with "Aluminon" which is stable under the same condition as the aluminum lake. If the iron content is small in proportion to the aluminum (1 : 10) and does not exceed 0.03–0.04 mg., it may be determined in a separate portion of the sample and equivalent amounts added to the standard Nessler tubes. For greater quantities of iron a separation must be made.

6. The color intensity decreases rapidly during the first twenty minutes after the addition of 5 cc. of 5 N ammonium hydroxide, and then remains practically constant for an hour or more, though it does decrease slowly. The time required for the analysis may be reduced considerably by the use of a solution of ammonium carbonate in ammonium hydroxide (250 grams ammonium carbonate in a liter of 5 N ammonium hydroxide). By the addition of 5 cc. of this ammonium hydroxide-carbonate solution the period of rapid decrease in color intensity is reduced to 15 minutes, but the constancy of the final color intensity is somewhat less dependable and the delicacy of the test is slightly decreased. With the exception of the ammonium carbon-

ate solution, small variations in the quantity of the reagents used produce marked differences in the color intensity.

7. The final color intensity varies with the temperature at which the lake forms, the intensity at 20° C. being four-fifths of that at 40° C. Hence, care should be taken to have the solution at the same temperature when the dye is added.

8. The final color intensity increases with the time allowed for lake formation, the intensity resulting from a 10-minute period being about 15 per cent higher than that obtained when a period of 5 minutes is given. The final color intensity is also a function of the volume at lake formation, and, hence, the volumes of both the sample and the standard must be the same at the time the lake is formed.

9. In the use of "Aluminon" as a qualitative test for aluminum, interference by chromium is prevented by an unusual property of the aluminum lake, which, when once formed in acetic acid-acetate buffer, is not decomposed in any reasonable time when the solution is made alkaline with ammonium hydroxide, although it does not form in alkaline solution. The chromium lake, which resembles the aluminum lake in appearance, forms in acetate solution, but is decomposed upon the addition of ammonium hydroxide. Under the conditions of the test (Hammett and Sottery, *loc. cit.*), (1) silicic acid and salts of bismuth, lead, antimony, stannic tin, mercuric mercury, and titanium give white precipitates; (2) salts of cadmium, zinc, manganese, cobalt, and nickel give no precipitates; (3) chromium, alkaline earth, and phosphate do not interfere if ammonium hydroxide-carbonate solution is added; (4) ferric salts give a deep violet precipitate in the acetic acid solution, but this is converted into a reddish-brown precipitate by ammonium hydroxide. The separation of iron from aluminum by sodium hydroxide and peroxide is sufficient to prevent the interference of iron.

10. Middleton<sup>3</sup> reports that "Aluminon" forms lakes with the hydroxides or basic acetates of beryllium, yttrium, lanthanum, cerium, neodymium, erbium, zirconium, and thorium. "All these are deeper red than the aluminum lake, the color being a rich bright crimson, that with cerous hydroxide much darker than the others. All, except that of beryllium, are either dissolved or decolorized by moderate additions of ammonium carbonate. Accordingly, the reagent does not distinguish aluminum from beryllium in mixtures of the two hydroxides.

<sup>3</sup> J. Am. Chem. Soc., 48, 2125 (1926).



The lakes are not affected by moderate concentrations of ammonia except that of zirconium which is partially decolorized and flocculates as a rose-pink precipitate. All are distinctly less soluble than the corresponding hydroxides or basic acetates."

Corey and Rogers<sup>6</sup> have studied the reactions of "Aluminon" with scandium, gallium, indium, thallium, and germanium. "Scandium produces a red lake, insoluble in ammonium hydroxide but readily soluble in ammonium carbonate. The gallium lake forms more slowly but, when once produced, more closely resembles that of aluminum, being insoluble in ammonium hydroxide and dissolving in a solution of ammonium carbonate only after standing for some time.

"Indium gives a red solution which is relatively stable in the presence of ammonium hydroxide, although no precipitate is produced. The red color of the solution is discharged by the addition of ammonium carbonate. In this respect it approaches the behavior of tervalent thallium whose lake forms with difficulty and is unstable both in ammonium hydroxide and in ammonium carbonate. Germanium forms no stable lake with 'Aluminon,' in this respect resembling silicon, stannic tin and lead."

**DETERMINATION OF ALUMINUM IN NON-FERROUS MATERIAL BY "ALUMINON" (THE AMMONIUM SALT OF AURIN TRICARBOXYLIC ACID)**

**METHOD OF LUNDELL AND KNOWLES**

Like the preceding method, this determination is based upon the use of the new reagent for aluminum, the dye aurin tricarboxylic acid recently described by Hammett and Sottery.<sup>7</sup>

The methods described in the following procedures were developed by Lundell and Knowles<sup>8</sup> for the rapid detection and approximate quantitative determination of small amounts of aluminum in non-ferrous materials such as brass, bronze, bearing metals, and spelter, even though the amount is as small as 0.01 per cent.

The aluminum must be separated from interfering elements and the most satisfactory rapid methods are those based on precipitation with sodium hydroxide or a mixture of this reagent with sodium sulfide. Three methods of separation are described. In the first, all the usual elements except aluminum and phosphorus are removed by precipita-

<sup>6</sup> J. Am. Chem. Soc., **49**, 216 (1927).

<sup>7</sup> J. Am. Chem. Soc., **47**, 142 (1925).

<sup>8</sup> Ind. Eng. Chem., **18**, 60 (1926).

tion with sodium hydroxide-sulfide reagent, filtration, acidification of the filtrate, and a second filtration. This method is the slowest of the three, but can be used with a greater variety of alloys. In the second method less sodium sulfide is added and the second filtration is omitted. This method requires only 5 to 10 minutes, and gives satisfactory results with most alloys provided care is taken to avoid adding so much sulfide as to cause the liberation of colored sulfides or hydrogen sulfide when the filtrate is acidified. The judicious use of sulfide aids in precipitating elements such as lead in lead-base bearing metal and gives a nearer complete separation of iron in material such as spelter. No sulfide is used in the third method, which otherwise resembles the second. This has given satisfactory results with brass and such other alloys as give a large precipitate with sodium hydroxide.

#### Reagents.

1. Nitric acid, sp. gr. 1.42.
2. Hydrochloric acid, 1 : 1.
3. Acetic acid, 36 per cent.
4. Ammonium carbonate, 10 per cent solution in dilute ammonium hydroxide (1 : 2). Prepare as needed, using freshly distilled ammonia.
5. Sodium hydroxide, 8 per cent.
6. Sodium sulfide, 8 per cent. Make from the sodium hydroxide solution by saturating with  $\text{H}_2\text{S}$  and then adding an equal volume of the sodium hydroxide.
7. Aurin tricarboxylic acid. Use a 0.2 per cent solution of "Aluminon" (the  $\text{NH}_4$  salt of aurin tricarboxylic acid). This reagent may be prepared by the second method of Ger. pat. 49,970 (1889), Friedländer II, 50, or may be obtained from the Fales Chemical Co., 74 Cortlandt St., New York City.

#### Procedures.

*Method I. Separation of all ordinary elements except aluminum and phosphorus.*

Transfer 1 gram of the alloy to a 250 cc. Erlenmeyer flask and dissolve in 5 cc. of concentrated nitric acid. Add 30 cc. of an 8 per cent solution of sodium hydroxide, and heat to boiling as the flask is rotated over a free flame. Boil for 1 minute and add 20 cc. of an 8 per cent solution of sodium sulfide made from the sodium hydroxide solution by

saturating a given volume with hydrogen sulfide and then adding another volume of sodium hydroxide. Swirl the solution for a few moments, and filter on a 15 cm. paper. Acidify the filtrate with dilute hydrochloric acid (1 : 1), add 2 cc. in excess, and digest at 40° to 60° C. until the precipitate settles. Filter, boil the filtrate until hydrogen sulfide is expelled, and clarify the solution by small additions of nitric acid if suspended sulfur is present. Evaporate the solution to 20 to 30 cc. and filter if the solution is not clear. Add 10 cc. of 36 per cent acetic acid, 5 cc. of a 0.2 per cent solution of the aurin tricarboxylic acid, and finally a 10 per cent solution of ammonium carbonate in dilute ammonium hydroxide (1 : 2) slowly and with stirring until an excess of 5 to 10 cc. is present. Avoid a large excess. Compare the color of the solution or the amount of separated lake with the results of tests of like material containing known amounts of aluminum.

*Method II. Single filtration after precipitation with NaOH-Na<sub>2</sub>S.*

Dissolve the alloy as in Method I, add 50 cc. of 8 per cent sodium hydroxide solution, heat to boiling, and boil for 1 minute. Add 8 per cent sodium sulfide solution in accordance with the material under test, swirl the solution for a few minutes, and filter. Add 15 cc. of acetic acid, 5 cc. of a 0.2 per cent solution of the dye, and then ammonium hydroxide-carbonate as in Method I.

More or less precipitate other than the aluminum lake may separate in the final test, but such precipitated matter should be white if a correct amount of sodium sulfide was added and should not obscure the color of the aluminum lake in alloys containing no more than 10 per cent of tin or 25 per cent of lead. Desirable additions of the sodium sulfide solution are: 0.5 cc. for tin-base bearing metal, 1 cc. for spelter, cast bronze, and phosphor bronze, 2 cc. for brass and journal bearing and 4 cc. for lead-base bearing metal.

*Method III. Single filtration after precipitation with NaOH.*

Proceed as in Method II, but omit the addition of sodium sulfide.

As in Method II, more or less white precipitate of tin and lead can be expected in the final test; this does not cause much trouble in brasses, bronzes, and journal bearings. With lead and tin-base bearing metals the method is not entirely satisfactory because the final precipitate is quite large. The method is not so certain for spelter as is Method II, because so little precipitate is formed by the sodium hydroxide alone that the precipitation of iron is sometimes incomplete.

**Notes.**

1. In all procedures the filtrations are continued until the filter papers are fairly well drained and no washing of the precipitate is done.

2. In each of the above procedures the final test for aluminum is always obtained in alkaline solution by adding a solution of ammonium carbonate in dilute ammonium hydroxide, slowly and with continual stirring, to a solution of the dye and free acetic acid. In such a test the final color of a solution of the dye alone is clear light yellow, but with aluminum the color ranges from a clear faint pink to a deep red if the amount of aluminum is not over 0.1 mg. in 50 cc. of solution. With larger amounts of aluminum the red lake precipitates at once. The use of the dye for a quantitative estimation is therefore limited to material containing less than 0.1 per cent of aluminum and works best when the percentage is less than 0.05.

3. The results given in the following table were obtained by Lundell and Knowles (*loc. cit.*) and indicate which method is the most suitable for a given type of alloy.

TABLE X

(Numbers Refer to Bureau of Standard's Standard Samples)

Material	Per Cent Al Present or Added	Character of the Test Obtained in:		
		Method I	Method II	Method III
Spelter.....	0.003	.....	Excellent	Good
	0.006	.....	Excellent	Good
Brass 37b.....	0.003	Excellent	Good	Good
	0.006	Excellent	Good	Good
Bronze 52.....	0.003	Excellent	Weak	Weak
	0.01	Excellent	Good	Fair
Phosphor bronze 63.....	0.04	Excellent	Good	Fair
Journal bearing.....	0.003	Excellent	Good	Fair
	0.01	Excellent	Good	Fair
Lead-base bearing metal 53..	0.003	Excellent	Unsatisfactory	Unsatisfactory
	0.006	Excellent	Very weak	Very weak
	0.01	.....	Fair	Weak
Tin-base bearing metal 54...	0.003	Good	Unsatisfactory	Unsatisfactory
	0.006	Good	Unsatisfactory	Unsatisfactory
	0.01	.....	Very weak	Very weak

4. In the use of "Aluminon" as a qualitative test for aluminum, interference by chromium is prevented by an unusual property of the aluminum lake, which, when once formed in an acetic acid-acetate buffer, is not decomposed in any reasonable time when the solution is made alkaline with ammonium hydroxide, although it does not form in alkaline solution. The chromium lake, which resembles the aluminum compound in appearance, forms in an acetate solution, but is decolorized upon the addition of ammonium hydroxide. Under the conditions of the test (Hammett and Sottery, *loc. cit.*) (1) silicic acid and salts of bismuth, lead, antimony, stannic tin, mercuric mercury, and titanium give white precipitates; (2) salts of cadmium, zinc, manganese, cobalt, and nickel give no precipitate; (3) chromium, alkaline earths, and phosphate do not interfere if ammonium hydroxide-ammonium carbonate solution is added; (4) ferric salts give a deep violet precipitate in the acetic acid solution but this is converted into a reddish-brown precipitate by ammonium hydroxide. The separation of iron from aluminum by sodium hydroxide or peroxide is sufficient to prevent the interference by iron. The delicacy of the test is of the order of  $10^{-6}$  mole of aluminum.

5. Middleton<sup>9</sup> reports that "Aluminon" forms lakes with the hydroxides or basic acetates of beryllium, yttrium, lanthanum, cerium, neodymium, erbium, zirconium, and thorium. "All these are deeper red than the aluminum lake, the color being a rich, bright crimson, that with cerous hydroxide much darker than the others. All, except that of beryllium, are either dissolved or decolorized by moderate additions of ammonium carbonate. Accordingly, the reagent does not distinguish aluminum from beryllium in mixtures of the two hydroxides. The lakes are not affected by moderate concentrations of ammonia except that of zirconium which is partially decolorized and flocculates as a rose-pink precipitate. All are distinctly less soluble than the corresponding hydroxides or basic acetates."

Corey and Rogers<sup>10</sup> have studied the reactions of "Aluminon" with scandium, gallium, indium, thallium, and germanium. "Scandium produces a red lake, insoluble in ammonium hydroxide but readily soluble in ammonium carbonate. The gallium lake forms more slowly but, when once produced, more closely resembles that of aluminum, being insoluble in ammonium hydroxide and dissolving in a solution

<sup>9</sup> J. Am. Chem. Soc., **48**, 2125 (1926)

<sup>10</sup> J. Am. Chem. Soc., **49**, 216 (1927).

of ammonium carbonate only after standing for some time. Indium gives a red solution which is relatively stable in the presence of ammonium hydroxide, although no precipitate is produced. The red color of the solution is discharged by the addition of ammonium carbonate. In this respect it approaches the behavior of trivalent thallium whose lake forms with difficulty and is unstable both in ammonium hydroxide and in ammonium carbonate. Germanium forms no stable lake with "Aluminon," in this respect resembling silicon, stannic tin and lead."

6. Since aluminum is one of the most common impurities in chemical reagents, it is necessary to be certain it is not present in any reagent employed in the analysis.

#### DETERMINATION OF ALUMINUM BY ALIZARIN-S

When the sodium salt of alizarin monosulfonic acid is added to a faintly acid solution of an aluminum salt, a red coloration or a precipitate is produced.<sup>11</sup> The presence of glycerine will prevent or retard the formation of a precipitate. The test is sensitive to one part of aluminum in ten million parts of solution.

##### Reagents.

1. Hydrochloric acid, 6 N.
2. Acetic acid, 6 N.
3. Ammonium hydroxide, 6 N. Made from freshly distilled ammonia as needed.
4. Glycerine, C.P.
5. Alizarin-S, 1 per cent solution.
6. Standard aluminum solution. Dissolve 0.9311 gram of common alum,  $\text{Al}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ , in water, dilute to a liter and thoroughly mix. Ten cc. of this solution are diluted to one liter and mixed thoroughly. The diluted solution contains 0.001 mg. of  $\text{Al}_2\text{O}_3$  per cubic centimeter.

**Procedure.**— A sample is taken such that its aluminum content is between 0.005 and 0.05 mg. and dissolved in the least possible quantity of dilute hydrochloric acid. In case stronger acid is required to bring the sample into solution, the excess acid is neutralized with ammonium hydroxide. The final volume of the solution should not exceed 25 cc., an aliquot part being taken if necessary. To the solu-

<sup>11</sup> F. W. Attack, J. Soc. Chem. Ind., **34**, 936 (1915).

tion, or an aliquot part, add 10 cc. of glycerine and 5 cc. of 1 per cent alizarin reagent, and dilute to about 40 cc. After mixing, allow the solution to stand 5 minutes and then acidify with acetic acid until no further change in color is observed, gently mixing after each addition of acid. Finally, dilute the solution to 50 cc. with water, mix, and compare at once with a standard prepared along with the sample under as near the same conditions as possible. The comparison is made by the method of dilution or balancing.

### Notes.

1. The color produced by adding alizarin reagent to dilute solutions of aluminum is not permanent and, hence, a series of natural standards is not satisfactory. On account of a slight fading that may occur before the matching can be made, the use of a set of artificial standards is not recommended. The method of duplication cannot be used on account of the time required to produce the color after the alizarin has been added to the standard.

2. A large excess of alizarin reagent is required and 5 minutes must be allowed for it to react with the alumina. After the addition of acetic acid, the comparison of color must be made as quickly as possible on account of the fact the acid gradually attacks the finely divided precipitate, especially in the presence of phosphates.

3. The colorimetric estimation of aluminum is not affected by moderate amounts of calcium, magnesium, and zinc salts, nor by relatively small quantities of phosphate, chromium, and iron. For example, 0.003 mg. of Al can be detected in the presence of 1 mg. of  $\text{Fe}^{+++}$  or 10 mg. of  $\text{Cr}^{+++}$ . Citric acid or a citrate may be used to keep larger amounts of iron in solution. If cobalt is present, an excess of ammonium hydroxide must never be used.<sup>12</sup>

4. Since aluminum is one of the most common impurities in chemical reagents, it is necessary to be certain it is not present in any reagent employed in the analysis. Freshly distilled ammonia must be used for preparing the ammonium hydroxide solution, and the latter prepared only as needed.

5. Nitric acid causes a more rapid fading of color than do the other acids and, hence, its use should be avoided as far as possible.

<sup>12</sup> Atack, *loc. cit.*

**DETERMINATION OF ALUMINUM BY HEMATOXYLIN****METHOD OF HATFIELD<sup>13</sup>**

This method is a modification of the hematoxylin or logwood test for aluminum and consists in forming the hematoxylin-aluminum color compound in samples of water which have been adjusted to *pH* 8.2 to 8.3, and then acidifying the color solution to *pH* 4.5. The method gives results accurate to 0.1 part of aluminum per million parts of water, and is well fitted for routine water analysis in that it is rapid and not interfered with by the alumino complexes present in raw water.

**Reagents.**

1. Acetic acid, 30 per cent. Dilute glacial acetic acid with distilled water.

2. Ammonium carbonate. Use a saturated solution. Keep the solution in a glass-stoppered bottle in the presence of an excess of solid ammonium carbonate.

3. Hematoxylin solution. Dissolve 0.1 gram of C. P. hematoxylin (white crystals) in 100 cc. of boiling distilled water. This solution is stable for 2 or 3 weeks.

4. Standard aluminum solution. Dissolve 0.8405 gram of ammonium alum,  $(\text{NH}_4)_2\text{SO}_4 \cdot \text{Al}_2(\text{SO}_4)_3 \cdot 24\text{H}_2\text{O}$ , in distilled water, dilute to a liter, and mix thoroughly. Dilute one cubic centimeter of this solution to 50 cc. One cubic centimeter of the diluted solution contains 1.0 p.p.m. aluminum. Use also a 1 : 10 dilution of the standard solution.

**Procedure.**—Place 50 cc. of the water to be tested in a 50 cc. Nessler cylinder (tall form), add 1 cc. of a saturated solution of ammonium carbonate and 1 cc. of hematoxylin solution. Mix by inverting the tube twice. Allow the solution to stand for 15 minutes in order to develop the maximum lavender color, then acidify with 1 cc. of 30 per cent acetic acid and compare the color with that of standard color tubes prepared in exactly the same way and at the same time by using standard ammonium alum solution and distilled water (see Note 3) to represent 0.0 to 1.0 p.p.m. aluminum.

**Notes.**

1. When the concentration is less than 0.15 p.p.m. aluminum the color is compared against white paper through the length of the Nessler

<sup>13</sup> Ind. Eng. Chem., **16**, 233 (1924).



cylinders, but with higher concentrations it is best to make the comparison through the sides of the tubes.

2. The unknown and standard color solutions should be made as near at the same time as possible, because the color varies with the time of formation. A difference of 15 minutes will make some difference in the tint, but will allow readings to 0.1 p.p.m. aluminum.

3. If color standards prepared with distilled water are unsatisfactory because of interfering ions, the standards should be made with the raw water which has been filtered through a Berkefeld filter. In this way the naturally occurring ions which interfere with the color reaction are compensated for in the standards. In using the Berkefeld filter care must be taken that it does not become contaminated with alum dust from the plant.

4. "Many comparisons of results obtained by the gravimetric method and the modified hematoxylin method have been made on filtered water samples with hydrogen-ion concentrations ranging from 3.3 to 9.0  $pH$  units. The comparisons show that the results check very satisfactorily where the  $pH$  values of the samples were above 6.0. Samples of the filtered water with  $pH$  values below 6.0 very rapidly dissolve silica from the ordinary flint glass laboratory sample bottles. This is particularly true as the acidity increases. Unless special precautions are taken to remove all silica, the gravimetric results on these acid samples are high. The modified hematoxylin method gave very consistent results on acid samples, which compared closely with the theoretical amount of aluminum ions added. Near the two extreme ends of the aluminum hydroxide insolubility zone ( $pH$  5.7 to 7.3) the hematoxylin method is more sensitive than the gravimetric method."<sup>14</sup>

<sup>14</sup> W. D. Hatfield, *loc. cit.*

## CHAPTER IX

### ANTIMONY AND ARSENIC

#### DETERMINATION OF ANTIMONY AS THE SULFIDE

This method<sup>1</sup> is especially adapted to the determination of antimony in alloys (copper, brass, etc.) in amounts varying between 0.003 and 0.1 per cent. The copper is removed by precipitation in  $\text{H}_2\text{SO}_4$  solution with  $\text{NaH}_2\text{PO}_2$ . Hydrochloric acid is then added to the filtrate and more  $\text{NaH}_2\text{PO}_2$  added. This precipitates the arsenic. The antimony is next precipitated by Cu as in the Reinsch test. The antimony is dissolved by treatment with  $\text{Na}_2\text{O}_2$ , a trace of  $\text{Cu}^{++}$  is precipitated by  $\text{ZnS}$ , the  $\text{SbO}_4^{---}$  reduced to  $\text{Sb}^{+++}$  by  $\text{SO}_2$ , the  $\text{SO}_2$  eliminated by boiling, and the solution finally saturated with  $\text{H}_2\text{S}$  after the addition of a little gum arabic solution. The resulting yellow colloidal suspension of  $\text{Sb}_2\text{S}_3$  is matched against a standard suspension.

#### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Sulfuric acid, 6 N and 9 N.
3. Nitric acid, 6 N.
4. Hydrochloric acid, sp. gr. 1.19.
5. Hydrochloric acid, 6 N.
6. Sodium hypophosphite,  $\text{NaH}_2\text{PO}_2$ .
7. Sodium peroxide.
8. Strip of metallic copper, about  $1.5 \times 10$  cm.
9. Zinc sulfide.
10. Sulfur dioxide.
11. Hydrogen sulfide. Use pure  $\text{H}_2\text{S}$  prepared as directed on p. 240.
12. Benzene.
13. Gum arabic solution, 1 per cent.
14. Standard antimony solution. Dissolve 0.2668 gram of potassium antimonyl tartrate,  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6$ , in hydrochloric acid and

<sup>1</sup> B. S. Evans, *Analyst*, **47**, 1 (1922).

dilute to one liter, adding a little more acid if needed to keep the antimony in solution. Mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of antimony.

**Procedure.**—Dissolve 5 grams of the alloy in 60 cc. of 6N  $\text{HNO}_3$  and 10 cc. of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84). Evaporate until dense white fumes are given off, cool, and dilute to 100 cc. Next add 14 grams of solid  $\text{NaH}_2\text{PO}_2$ , heat the solution almost to boiling, filter through a rapid filter into a 750 cc. flask, and wash the spongy copper several times with hot water. Add 2 grams more of  $\text{NaH}_2\text{PO}_2$ , 100 cc. of  $\text{HCl}$  (sp. gr. 1.19), and boil 15 minutes to precipitate the arsenic. Add more  $\text{NaH}_2\text{PO}_2$  if needed to complete the precipitation of the arsenic. Finally add 10 cc. of benzene, which will serve to condense the colloidal arsenic, and filter through a filter previously wetted with water. The mixture of colloid and benzene will not pass through the wet filter. Test the filtrate by boiling to see if more arsenic precipitates. Should arsenic still be present, add more  $\text{NaH}_2\text{PO}_2$ . To the final filtrate, after complete removal of arsenic, add a strip of copper foil, about  $1.5 \times 10$  cm., which has been coiled into a flat spiral and cleaned by heating in 6 N  $\text{HNO}_3$  and washing with water. Gently boil the solution 1.5 to 2 hours. The antimony precipitates on the copper foil. Decant the solution, wash the antimony deposit rapidly with cold water, and quickly place it in a beaker, cover with water and add 1 gram of  $\text{Na}_2\text{O}_2$ . Heat until the deposit has apparently dissolved and decant the solution. Wash the copper foil several times with cold water. It will be tarnished with a little  $\text{CuO}$ . Cover it with 6 N  $\text{HCl}$ . This will remove the stain due to  $\text{CuO}$ . If the coil is not bright after immersion in  $\text{HCl}$ , repeat the treatment with  $\text{Na}_2\text{O}_2$  in order to remove the remaining small quantity of antimony. To the solution containing the antimony add 0.5 gram of  $\text{ZnS}$  and let stand about 2 hours to insure the precipitation of the last trace of copper as  $\text{CuS}$ . Then filter, wash the precipitate with a little cold water, acidify with  $\text{HCl}$  and introduce  $\text{SO}_2$  to reduce  $\text{SbO}_4^{---}$  to  $\text{Sb}^{+++}$ . This should require only 3 to 5 minutes. Boil gently until every trace of  $\text{SO}_2$  has been driven off. It is advisable to evaporate to a volume of 10 cc. Measure out 5 cc. of the standard antimony solution, add 80 cc. of water, reduce with  $\text{SO}_2$  and concentrate as in the solution analyzed. Cool both solutions, add to each 5 cc. of a 1 per cent gum arabic solution, and pass  $\text{H}_2\text{S}$  into them until the maximum color develops. This will require only a few seconds. Transfer the solutions to Nessler

tubes and dilute the stronger one until it matches the other. By measuring the height of each solution, the quantity of antimony is easily calculated.

#### Notes.

1. The gum arabic solution is added to keep the  $\text{Sb}_2\text{S}_3$  in colloidal suspension.

2. If the alloy contains a large amount of tin, low results will be obtained due to the precipitation of antimony with the metastannic acid gel. In order to prevent this, add 5 grams of  $\text{KHC}_4\text{H}_4\text{O}_6$  after diluting the evaporated  $\text{H}_2\text{SO}_4$  solution. The  $\text{KHC}_4\text{H}_4\text{O}_6$  will keep the antimony in solution but prevents the copper precipitating as easily as it would otherwise. Hence, it is necessary to add 14 grams of  $\text{NaH}_2\text{PO}_2$ .

3. The presence of bismuth does not interfere with the analysis.

#### REFERENCES

1. P. Schidrowitz and H. A. Goldsbrough, *Analyst*, **36**, 101 (1911).
2. W. Beam and G. A. Freak, *ibid.*, **44**, 196 (1919).
3. B. S. Evans, *ibid.*, **47**, 1 (1922).

#### DETERMINATION OF ARSENIC BY FORMATION OF A STAIN ON MERCURIC BROMIDE PAPER BY ARSINE

The method depends upon the reduction of arsenates first to arsenious acid and finally to arsine. The arsine is then passed over strips of paper impregnated with mercuric bromide and the stains produced on the paper are compared with stains produced by known amounts of arsenic.

#### Reagents.

1. Hydrochloric acid, 3 N.
2. Sulfuric acid, 6 N.
3. Ferrous sulfate, C. P.
4. Potassium iodide, C.P.
5. Stannous chloride. Saturate hydrochloric acid solution (sp. gr. 1.2) with tin, dilute with an equal volume of water, and add a slight excess of acid from time to time. Keep a strip of metallic tin in the bottle.

6. Lead acetate. A 5 per cent solution and also strips of lead acetate paper.

7. Zinc. Arsenic-free.

8. Mercuric bromide paper. Soak heavy strips (2.5 cm.  $\times$  12 cm.) of drafting paper in a 5 per cent solution of mercuric bromide for an hour, remove the strips, squeeze out the excess of solution, and hang them up to dry.

9. Standard arsenic solution. Dissolve 0.1320 gram of pure arsenious oxide,  $As_2O_3$ , in 2.5 N sodium hydroxide, add several hundred cubic centimeters of water, acidify with hydrochloric acid, and dilute to a liter. Thoroughly mix. Ten cubic centimeters of this solution are diluted to 250 cc. One cubic centimeter of the diluted solution contains 0.004 mg. of arsenic.

10. Standard arsenic stains. A series of stains is prepared in the same way as that specified for the sample (see procedure below). The arsenic should vary at intervals from 0.001 to 0.05 mg.

**Procedure.**—Unless the sample contains iron, the latter must be added (in the form of a ferrous salt) before the reduction of the arsenic.<sup>2</sup> The reduction of arsenate to arsenious acid is obtained by heating almost to boiling the sample in 3 N HCl with KI and a few cubic centimeters of stannous chloride. The arsenic content of the sample must be less than 0.04 mg.

The generator consists of a small (1 or 2 oz.) wide-mouth bottle fitted with an upright tube about 15 cm. long. The tube is divided into two chambers; the upper is filled with cotton moistened with lead acetate solution, the lower is filled with strips of lead acetate paper. A strip of the mercuric bromide paper is suspended in a capillary tube (3 mm.  $\times$  12 cm.) which is attached to the top of the 15 cm. tube. A "blank" run for 1 hour is made with 40 cc. of 6 N sulfuric acid, 5 drops of the stannous chloride solution, and about 10 grams of arsenic-free zinc. The sample, reduced as mentioned above, is then introduced into the generator. The arsine evolved comes in contact with the mercuric bromide paper in the capillary and produces a deep orange stain of varying length. The stain shades sharply to a light yellow and then to the untouched paper. The stained paper is matched against a series of stains produced by known amounts of arsenic.

<sup>2</sup> W. S. Allen and R. M. Palmer, *Orig. Com. 8th Intern. Cong. Appl. Chem.*, **1**, 9 (1912); see also U. S. Dept. of Agriculture, Bureau of Chem., Bulletin No. 102.

### Notes.

1. Sulfides, compounds of antimony, and phosphoric acid interfere with the analysis due to the formation of hydrogen sulfide, stibine, and phosphine, respectively. The arsenic may be separated from antimony by precipitation with magnesium phosphate, but this is not very satisfactory. It is better to concentrate a large amount of the substance and estimate the arsenic gravimetrically. Sulfides and phosphoric acid may be removed by oxidation with nitric acid. Fluorine, if present in more than a trace, must be removed from the sample.

2. The purpose of the stannous chloride solution in the reduction of arsenates to arsenious acid is to take up any iodine liberated during the reduction.

3. Arsenates may be reduced to arsenious acid by the action of nascent hydrogen. This method, however, is difficult and it is better to reduce the arsenate before adding the sample to the generator.

4. The purpose of the lead acetate in the generator tube is to remove traces of hydrogen sulfide which would stain the mercuric bromide paper.

5. It is necessary to run a "blank" in order to be certain that arsenic is not introduced by any of the reagents.

6. The series of standard stains are prepared in the same way as the stain from the sample and will keep for six months if made with mercuric bromide papers. Mercuric chloride papers can be used but the standard stains will fade in a few days.

7. The length of the orange stain varies with the amount of arsenic present. If antimony is present the stain is longer and of a lighter shade of color than the standard. A stain due to antimony will fade when exposed to hydrochloric acid fumes, while an arsenic stain is intensified.<sup>3</sup>

8. The purpose of adding stannous chloride to the generator is to sensitize the zinc.

9. Arsenic-free iron is recommended for use in the generator in place of zinc.<sup>4</sup> The former produces arsine but not the undesired stibine or phosphine.

10. The generator must be adjusted so as to give a uniform rate of production of hydrogen during the formation of the sample stain and

<sup>3</sup> Disc. 8th Intern. Cong. Appl. Chem., **27**, 4 (1912).

<sup>4</sup> Cf. F. D. Snell, *Colorimetric Analysis*, p. 66. D. Van Nostrand Co., New York, 1921.

those of the standards. Any organic matter in the sample must be so treated that it will not interfere with the desired rate of flow of hydrogen.

#### DETERMINATION OF ARSENIC BY SILVER NITRATE<sup>5</sup>

This is a modification of the mercuric bromide method, the only difference being that a few crystals of silver nitrate are put in the capillary tube in place of the mercuric bromide paper. A black stain is produced on the crystals by the arsine and is compared with standard stains produced by known amounts of arsenic in a similar way. A series of standard tubes, representing varying amounts of arsenic, may be prepared and sealed. These will keep indefinitely.

#### DETERMINATION OF ARSENIC BY QUININE ARSENOMOLYBDATE

The method is based upon the colloidal suspension produced by adding quinine arsenomolybdate reagent to a solution containing pentavalent arsenic.

##### Reagents.

1. Molybdenum trioxide solution. Dissolve 3.5 grams of  $\text{Na}_2\text{CO}_3$  in 50 cc. of water, add 9.5 grams of  $\text{MoO}_3$ , heat on the water-bath till solution is complete, cool, dilute to 100 cc., and mix thoroughly.

2. Nitric acid. Dilute 25 cc. of  $\text{HNO}_3$  (sp. gr. 1.34) to 100 cc.

3. Standard arsenic solution. Add 1.3202 grams of pure  $\text{As}_2\text{O}_3$  to dilute  $\text{HNO}_3$ , evaporate on a water-bath nearly to dryness, dilute to a liter, and mix thoroughly. Ten cubic centimeters of this solution are diluted to a liter and thoroughly mixed. One cubic centimeter of the diluted solution contains 0.01 mg. of arsenic.

4. Quinine arsenomolybdate reagent. Dissolve 0.5 gram of *neutral* quinine hydrochloride in 10 cc. of water, add 5 cc. of (3) and 10 cc. of (2), and<sup>6</sup> then 1 cc. of (1), drop by drop with continuous stirring. The addition of reagent (1) forms a precipitate which almost completely redissolves in a few minutes, giving a slightly opalescent mixture. This is diluted to 120 cc., mixed, and filtered through a filter that has been washed with dilute  $\text{HNO}_3$  and then with water.

**Procedure.**—A sample is taken having an arsenic content between 0.008 and 0.035 mg. In the absence of  $\text{H}_2\text{S}$  group metals, the solution

<sup>5</sup> L. Moreau and E. Vinet, *Compt. rend.*, **158**, 869 (1914).

of the sample is strongly acidified with acid, saturated with  $\text{H}_2\text{S}$ , stoppered, and allowed to stand 24 hours. Then filter through asbestos, wash, dissolve the precipitate in hot concentrated  $\text{HNO}_3$  containing bromine water, and evaporate the filtrate and washings to dryness on the water-bath. The residue is taken up in 20 cc. of the quinine arsenomolybdate reagent. At the same time a standard is prepared by adding 0.5 cc. of (2) to 2.5 cc. of (3), then 2 cc. of water, and 20 cc. of (4). After the solutions have stood 15 minutes they are compared in a colorimeter. If desirable, the sample can be compared with a series of standards representing varying amounts of arsenic.

#### Notes.

1. Organic matter interferes with the analysis and must be destroyed by (1)  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ , (2)  $\text{HCl}$  and  $\text{KClO}_3$ , or (3) by igniting with  $\text{CaO}$  or with  $\text{MgO}$  and  $\text{Mg}(\text{NO}_3)_2$ .

2. Interfering metals are removed by means of  $\text{H}_2\text{S}$  or by distilling the arsenic as  $\text{AsCl}_3$ . *Procedure when  $\text{H}_2\text{S}$  group metals are present.*—After destroying the organic matter with  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ , most of the  $\text{H}_2\text{SO}_4$  is driven off, the cooled residue (less than 1 cc.) taken up with water, transferred to a 50 cc. glass-stoppered retort, and a little  $\text{KCl}$ , a little  $\text{KBr}$ , and a few crystals of hydrazine sulfate are added. The neck of the retort is slightly inclined upwards, and is connected to a vertical bulbed tube, the end of which has been drawn out to a fine opening, the latter dipping into a few cubic centimeters of water. Carefully distill the solution nearly to dryness, cool, add 1 cc. conc.  $\text{HCl}$ , again distill almost to dryness, and repeat 3 or 4 times. Add to the distillate 20 to 30 cc. of water, 5 cc. of concentrated  $\text{HNO}_3$ , 5 cc. of bromine water, and evaporate to dryness on the water-bath. The residue is treated with 20 cc. of the quinine arsenomolybdate solution and after standing 15 minutes is compared with a standard prepared as described in the Procedure.

3. If the organic matter was destroyed by igniting with  $\text{CaO}$ , or  $\text{MgO}$  and  $\text{Mg}(\text{NO}_3)_2$ , the mass is dissolved in the least possible amount of  $\text{HCl}$  and water, and the distillation carried out as directed in Note 2.

4. If antimony or mercury is present, the temperature must be kept below  $115^\circ \text{C}$ . to prevent them from passing over into the distillate. Should this happen, they would cause loss of arsenic in the subsequent evaporation.



5. This method has been tested by Chouchak<sup>6</sup> using solutions of known arsenic content, both alone and after the addition of copper, mercury, antimony, and organic matter (sugar, wine), and excellent results were obtained. With amounts of arsenic varying between 0.00257 and 0.0429 mg. the errors were between 0.0001 and 0.0012 mg.

<sup>6</sup> Ann. chim. anal. chim. appl. [2], 4, 138 (1922).

## CHAPTER X

### BISMUTH, BORON, AND BROMINE

#### DETERMINATION OF BISMUTH AS THE IODIDE

##### METHOD A

WHEN potassium iodide is added to a dilute nitric or sulfuric acid solution containing a small amount of bismuth a yellow color is produced, due to the formation of bismuth iodide. The depth of color is proportional to the bismuth content of the solution. The method is suitable for the determination of bismuth in small quantities. The weight of bismuth in the sample taken for analysis must not be over 0.15 mg.<sup>1</sup>

##### Reagents.

1. Nitric acid, 6 N.
2. Sulfurous acid. Use a saturated solution.
3. Sodium carbonate, 3 N.
4. Ammonium carbonate, 3 N.
5. Ammonium hydroxide, 6 N.
6. Lead nitrate, 13.5 grams per liter.
7. Potassium iodide. Dissolve 17 grams in water and dilute to a liter.
8. Standard bismuth solution. Dissolve 0.2321 gram of bismuth nitrate,  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ , in dilute nitric acid, dilute to a liter and thoroughly mix. Or, a similar standard may be made by dissolving 0.1 gram of pure bismuth in nitric acid and diluting to a liter, care being taken to have sufficient acid present to prevent the bismuth precipitating as the sub-nitrate. Each of these solutions contain 0.1 mg. of bismuth per cubic centimeter. For the dilution or balancing methods the standard is further diluted as follows: Add 10 cc. of concentrated nitric acid to 50 cc. of the standard, dilute with about 800 cc. of water, add 25 cc. of potassium iodide solution and 5 cc. of sulfurous

<sup>1</sup> Eng. Mining J., 104, 1091 (1917).

acid, dilute to a liter and mix thoroughly. This solution contains 0.005 mg. of bismuth per cubic centimeter.

**Procedure.**—A sample is taken such that the bismuth content is not over 0.15 mg. and, if a solid, is dissolved in a small quantity of nitric acid. Sodium carbonate is then added to the solution of the sample. Bismuth, together with other metals, is precipitated and filtered. Test the filtrate to see if all of the bismuth was precipitated. If not, then more time must be given to allow the precipitate to form. In exceptional cases this may require 5 hours. Thoroughly wash the precipitate, dissolve in nitric acid, add 5 cc. of lead nitrate solution, nearly neutralize the solution with ammonium hydroxide and add ammonium carbonate in slight excess. Bismuth sub-nitrate precipitates almost free from other metals. Dissolve the bismuth sub-nitrate in nitric acid, taking care to thoroughly wash all parts of the filter with the acid. The volume is adjusted to about 25 cc. (by evaporation or dilution), 10 drops of sulfurous acid and 5 cc. of the potassium iodide solution are added and the solution diluted to a liter. Thoroughly mix. The color of the solution is then compared with a standard bismuth solution. Any of the four methods of comparison may be used.

#### Notes.

1. The solution must be free from large amounts of lead, copper, tin, antimony, gold, and silver.<sup>2</sup> These metals are removed by precipitation. Much copper, arsenic, and iron cause high results due to the liberation of iodine. Ammonium chloride, hydrochloric acid, and large amounts of antimony give low results.

2. The precipitation of bismuth may be hastened by thorough stirring or shaking.

3. In case the precipitate of bismuth sub-nitrate seems too impure it is redissolved in nitric acid and reprecipitated. If copper is present, as indicated by the color of the solution, a few cubic centimeters of potassium cyanide solution are added to decolorize the solution before the reprecipitation.

#### REFERENCES

1. P. Planes, *J. pharm. chim.*, **18**, (vi), 385 (1903).
  2. H. W. Rowell, *J. Soc. Chem. Ind.*, **27**, 102 (1908).
- <sup>2</sup> H. W. Rowell, *J. Soc. Chem. Ind.*, **27**, 102 (1908).

3. H. A. B. Motherwell, *Eng. Mining J.*, **104**, 1091 (1917).
4. W. T. Phillips, *ibid.*, **105**, 882 (1918).
5. G. Spurge, *Chem. Eng. Mining Rev.*, **11**, 80 (1918).

### METHOD B

This method is based upon Aubry's iodoquinic reagent which gives an orange-red color to dilute solutions of bismuth in acetone.

#### Reagents.

1. Nitric acid, 10 per cent.
2. Iodoquinic reagent (Aubry's reagent). One gram of quinine sulfate, 3 or 4 drops of sulfuric acid, and 2 grams of potassium iodide are dissolved in 100 cc. of water.
3. Standard bismuth solution. Use same as that in Method A.

**Procedure.**—A 10 cc. solution of the sample (Bi content between 0.1 and 1 mg.) in 10 per cent nitric acid is mixed with 2 cc. of the iodoquinic reagent and 8 cc. of acetone, and the resulting color matched against that of a standard prepared similarly.

**Note.**—Cuny and Poirot make the analysis in water solutions to which has been added gum arabic. The latter keeps the bismuth precipitate in colloidal suspension. Their results vary from  $-3.5$  per cent to  $+2.4$  per cent of the theoretical. The sources of error are: (1) Free acids, except  $\text{HNO}_3$  as used in the procedure and (2) several metal salts (especially iron salts) which set iodine free. In most cases the gum arabic prevents precipitation, but the salts may cause flocculation. The sample and standard should be prepared under as near the same conditions as possible.

#### REFERENCES

1. P. Aubry, *J. pharm. chim.*, **25**, 15 (1922).
2. L. Cuny and G. Poirot, *ibid.*, **28**, 215 (1923).
3. C. E. Laporte, *ibid.*, **28**, 304 (1923).

### DETERMINATION OF BORIC ACID BY TURMERIC PAPER

In this method the boric acid in the sample is treated with methyl alcohol, the resultant methyl borate distilled into an alkali solution and, after further treatment, the stain made on a standard turmeric paper is matched against a series of stains similarly produced with known amounts of boric acid. Or, the methyl borate distillate may be

evaporated to dryness, the residue taken up in a little water and hydrochloric acid, an ethyl acetate solution of turmeric added, and the resulting red color matched against a standard prepared by treating in the same way a solution containing a known quantity of boric acid.

#### Reagents.

1. Hydrochloric acid, sp. gr. 1.162.
2. Phosphoric acid.
3. Sodium carbonate, 1 N.
4. Methyl alcohol.
5. Turmeric paper. Select a good grade of drafting paper of uniform thickness and cut it into narrow strips (say 2 or 3 mm.). The papers are then soaked in a solution of turmeric, the excess of solution squeezed out, and the papers dried. The saturation must be made as uniform as possible.
5. Turmeric solution. Shake an excess of turmeric powder with 95 per cent ethyl alcohol, and filter the mixture.
6. Standard borate solution. Dissolve 0.2739 gram of sodium tetraborate,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , in water, dilute to a liter, and mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of  $\text{B}_2\text{O}_3$ .

#### THE USE OF TURMERIC PAPER

**Procedure.**—A sample of suitable size (see Note, p. 135) is placed in a platinum dish together with a little sodium carbonate and ignited until all organic matter has been destroyed. The ash is then treated with 5 to 10 cc. of phosphoric acid, the mixture poured into a small distillation flask, the dish rinsed out with 20 cc. of methyl alcohol and the washings added to the flask. Connect the flask to a condenser and distill from a water-bath, collecting the distillate in a platinum crucible containing a few drops of the sodium carbonate solution. Ten cubic centimeters more of methyl alcohol are added to the flask and the distillation again made, the distillate being collected in the crucible with the first one. Evaporate the combined distillates to dryness, cool, add 4 drops of hydrochloric acid and 0.5 cc. of water, transfer the solution to a small vial 30 mm. high (rinsing several times with 2 or 3 drops of water), and dilute to 1.5 cc. as shown by a mark on the vial. A strip of the turmeric paper ( $45 \times 3$  mm.) is then immersed

in the liquid 15 mm. and allowed to soak 3 hours at a temperature of 35° C. or 10-24 hours at room temperature. The height in millimeters of the red color thus produced is compared against a series of standard papers prepared by soaking strips of turmeric paper in solutions containing known amounts of borate treated in the same manner as the sample.

#### THE USE OF TURMERIC SOLUTION

**Procedure.**—The method of procedure is the same as directed above, except that the residue obtained from evaporation of the distillate is dissolved in 1 cc. of water and 2 cc. of hydrochloric acid (sp. gr. 1.162). One cubic centimeter of the ethyl acetate solution of turmeric is then added to the sample and to each of the standards similarly treated and containing known amounts of  $B_2O_3$ . The resulting red colors are compared after standing 50 minutes.

**Note.**—The size of sample taken for analysis depends upon the amount of borate suspected. Usually 1 gram is sufficient in case the sample is of vegetable origin, while 10 grams or more are ordinarily taken in case it is of animal origin.

#### REFERENCES

1. G. Bertrand and H. Agulhon, *Bull. soc. chim.*, **7**, 90, 125; *Ann. chim. anal. appl.*, **15**, 45, 89; *Compt. rend.*, **157**, 1433; *ibid.*, **158**, 201; *Bull. soc. chim.*, **15**, 292; *Bull. sci. pharmacolog.*, **21**, 68.
2. G. Halphen, *Ann. fals.*, **8**, 1 (1915); *J. Soc. Chem. Ind.*, **31**, 278.

#### DETERMINATION OF BORIC ACID BY CURCUMIN <sup>3</sup>

The method is based upon the red color produced when boric acid is added to a solution of curcumin containing oxalic acid. It is especially adapted to the estimation of boric acid present in food as an adulterant.

#### Reagents.

1. Hydrochloric acid, 6 N.
2. Oxalic acid. Saturated solution.
3. Barium hydroxide. Saturated solution.
4. Curcumin. Dissolve 0.5 gram in 500 cc. of alcohol.

<sup>3</sup>C. E. Cassal and H. Gerrans, *Chem. News*, **87**, 27 (1903); cf. Snell, *Colorimetric Analysis*, p. 126. D. Van Nostrand Co., New York, 1921.

5. Alcohol, 95 per cent.

6. Standard borate solution. Dissolve 0.2739 gram of sodium tetraborate,  $\text{Na}_2\text{B}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ , dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of  $\text{B}_2\text{O}_3$ .

**Procedure.**—Place the sample in a platinum or porcelain dish, make strongly alkaline with barium hydroxide, evaporate to dryness on a paraffin-bath at  $105^\circ \text{C}$ ., acidify the residue with hydrochloric acid and extract with several small portions of hot water. Filter the combined extracts into a 100 cc. flask. Transfer the filter and its contents to a platinum dish, make alkaline with barium hydroxide, and ignite in a Bunsen flame till the carbon has been removed. Dissolve the ash in a little hydrochloric acid, add the solution to the filtrate in the 100 cc. flask, dilute to the mark, and mix thoroughly. Pipette off 10 cc. of this solution, mix it in a porcelain dish with 10–15 grams of purified sand, make alkaline with barium hydroxide, and evaporate to dryness on a paraffin-bath, stirring from time to time to insure thorough mixing. Acidify the residue with hydrochloric acid, add 2 cc. of the oxalic acid solution, 2 cc. of the curcumin solution, mix thoroughly, and cover the dish with a large glass funnel (which extends well over the edge of the dish) whose stem is connected to a set of potash bulbs half filled with barium hydroxide solution. The potash bulbs are placed in cold water and connected to an aspirator. The dish is again heated on the paraffin-bath to dryness, this time a current of air being slowly drawn through the potash bulbs by suction. Add 1 cc. of the curcumin solution to the residue and again heat to dryness. The dry mass is now extracted with several small portions of alcohol, the solution in the potash bulbs added to the residue, and the mixture evaporated to dryness. Acidify the residue with hydrochloric acid, treat it with the same reagents as used with the original substance, heat to dryness and extract with several small portions of alcohol. Add this extract to the previous one, filter, and match the color of the solution against a standard by the method of dilution or balancing. The standard is prepared by treating 10 cc. of the standard borate solution in the same manner and along with the sample.

#### Notes.

1. In case the final solutions of the sample and standard do not match in tint, one having more of an orange tint than the other, it is due to an excess of curcumin. Add one or two drops of the curcumin

solution until similar tints are obtained and match by dilution or balancing.

2. The purpose of mixing sand with an aliquot part of the solution is to secure complete and intimate contact between the reacting substances at the drying-point (which is the point of reaction).

3. The use of the funnel and potash bulbs is to prevent small losses of boric acid due to evaporation. Free boric acid in solution is far more easily lost by evaporation than seems to have been generally supposed.

4. The second treatment of the ash from the 10 cc. aliquot part is usually sufficient to insure all the boric acid having entered into reaction.

#### DETERMINATION OF BROMINE \*

This method consists essentially in liberating the bromine from its salts by means of chlorine-water, dissolving the free bromine in carbon tetrachloride and matching the resulting yellow to reddish-brown solution thus obtained with a series of standard solutions similarly prepared with known amounts of sodium bromide. While the procedure was developed primarily for commercial natural brines, it will doubtless also apply to the analysis of artificial brines, such as those used in the manufacture of soda ash and in electrolytic cells.

##### Reagents.

1. Sulfuric acid, 1 N.
2. Sodium carbonate, 1 N.
3. Chlorine-water. Use a saturated solution.
4. Carbon tetrachloride.
5. Standard bromide solution. Dissolve 6.4390 grams of pure sodium bromide in water and dilute to a liter. Thoroughly mix. One cubic centimeter of the solution contains 5 mg. of bromine (as bromide). Dilute 50 cc. of this solution to 500 cc. with water and mix thoroughly. The diluted solution contains 0.5 mg. of bromine (as bromide) per cubic centimeter.

**Procedure.**—Place 100 cc. of the brine in an evaporating dish, add sodium carbonate until the liquid is alkaline and evaporate to dryness. Dissolve the residue in a little water and filter into a 250 cc. volu-

\* O. R. Sweeney and J. R. Withrow, *J. Ind. Eng. Chem.*, **9**, 674 (1917).



metric flask. Add sulfuric acid until the solution is distinctly acid, dilute to the mark and mix thoroughly. Place 25 cc. of the solution in a 50 cc. Nessler cylinder, add chlorine-water until the maximum depth of color develops, then add 10 cc. of carbon tetrachloride and shake thoroughly. Compare the resulting carbon tetrachloride solution of bromine with a set of standards made in a similar way, using known amounts of sodium bromide. This comparison will give the approximate amount of bromine in the sample solution. Then prepare a set of standards containing bromine very close above and below the amount estimated to be in the sample. Take another 25 cc. portion of the sample, add chlorine-water until the maximum depth of color develops and add the same amount to each of the standards. Shake the sample with 10 cc. of carbon tetrachloride, pour onto a wet filter and when the water has drained off, puncture the filter and catch the liquid in a 25 cc. Nessler cylinder. Match the color against a set of standards similarly prepared.

#### Notes.

1. It is best to make the final extraction, filtering, etc., in a darkened room, but this is not essential.
2. In case the sample does not exactly match the standard, the darker solution may be diluted with carbon tetrachloride; or, since the operation is so simple, a new set of standards may be prepared.
3. If a test shows that not all of the bromine was extracted by the 10 cc. of carbon tetrachloride a second extraction must be made. Usually this will not be necessary.
4. Traces of iodine present in most brines do not interfere and the iodine need not be reported.

## CHAPTER XI

### CALCIUM

#### DETERMINATION OF CALCIUM BY ALIZARIN<sup>1</sup>

THIS method depends upon the insolubility of calcium alizarinate. The calcium is first precipitated as the oxalate, then converted into the alizarinate under well-defined conditions, the latter filtered off, decomposed by oxalic acid, and the liberated alizarin dissolved in alcohol, the solution made just alkaline with ammonia and diluted to a definite volume. The resultant solution of ammonium alizarinate is matched in color against a similar solution as standard.

#### Reagents.

1. Hydrochloric acid 1 N.
2. Oxalic acid.
3. Ammonium hydroxide, sp. gr. 0.90.
4. Ammonium oxalate. A saturated solution and a 0.1 per cent solution.
5. Sodium acetate. Make a saturated solution and add a little ammonium oxalate to precipitate any calcium present.
6. Alcohol, 95 per cent.
7. Alizarin reagent. Dissolve 1 part of pure alizarin in 1000 parts of 95 per cent alcohol.
8. Standard alizarin solution. Prepare accurately a 0.001 molar solution of pure alizarin in 95 per cent alcohol. Two cubic centimeters of this solution are placed in a 50 cc. volumetric flask and 1 cc. of a strong solution of oxalic acid in 50 per cent alcohol is added together with a volume of 95 per cent alcohol approximately equal to the amount used in extracting the alizarin from the Gooch filter when dealing with the sample. Add water, make the solution just alkaline with ammonia, dilute to the mark, and thoroughly mix. The solution is

<sup>1</sup> P. P. Laidlaw and W. W. Payne, *Biochem. J.*, **16**, 494 (1922).

then transferred to the colorimeter (Duboscq used by L. and P., *loc. cit.*) and a similar solution of the sample matched against it.

**Procedure.**—A sample containing a few tenths (or less) of a milligram of calcium is taken for the analysis and carefully incinerated or sometimes, as in the case of blood serum, it may be used directly. Dissolve the ash in 0.5 cc. of N HCl and transfer to a centrifuge tube, using about 2 cc. of wash-water. Then add 1 cc. of a saturated solution of ammonium oxalate, 2 cc. of a saturated solution of sodium acetate (calcium free), mix thoroughly, and let stand for 3 hours, or over night if more convenient. The precipitated calcium oxalate is then centrifuged (2 min. at 4000 r.p.m. is sufficient) to the bottom of the tube, the supernatant liquid sucked off as completely as possible without disturbing the precipitate, 3 cc. of a 0.1 per cent solution of ammonium oxalate added, the precipitate stirred up and the centrifugation repeated at once. Again remove the supernatant liquid as completely as possible without disturbing the precipitate. Dissolve the precipitate of calcium oxalate in 0.5 cc. of N HCl and transfer the solution to a test tube, washing out the centrifuge tube four or five times with water. The total volume of the diluted solution in the test tube should be between 8 and 10 cc. Add an excess of alizarin reagent (1 cc. per 0.1 mg. of calcium), warm the solution on a water-bath to about 80° C., add five drops of concentrated ammonium hydroxide solution and mix. The mixture turns a deep purple. It is kept warm for about an hour and then set aside to cool and stand till the next day. All of the calcium should then have precipitated in clumps of blue-black microcrystalline needles of calcium alizarinate at the bottom of the test tube and the supernatant liquid should be pale in color.

The calcium alizarinate is filtered through a Gooch filter of fine asbestos, using gentle suction, and washed with dilute ammonia. The test tube in which the crystallization took place is put under the delivery from the Gooch crucible. Now pour over the precipitate 1 cc. of a strong solution of oxalic acid in 50 per cent alcohol, being careful to cover every part of the filter. The calcium alizarinate is at once decomposed and the color changes from the blue-black to orange. The alizarin thus liberated is washed through the filter (along with the excess of oxalic acid) with warm 95 per cent alcohol into the test tube. The alizarin solution is then transferred to a 50 cc. volumetric flask, the test tube being washed out with dilute ammonia. Make the solu-

tion just alkaline with ammonia, dilute to the mark with water, thoroughly mix, and transfer to the colorimeter for comparison against a standard solution of ammonium alizarinate. The alizarin in the sample is calculated from the height of the column of solution which matches the standard, and this figure divided by six gives the weight of the calcium.

#### Notes.

1. A series of determinations of the composition of calcium alizarinate, prepared under the conditions of the above procedure, shows that the calcium is only one-sixth of the weight of alizarin. Hence, to obtain the weight of calcium present in the sample it is only necessary to divide the weight of alizarin found by six.

2. Using calcium-free reagents and pure alizarin, and avoiding undue haste, 0.1 mg. of calcium may be estimated to 0.002 or 0.005 mg. without much trouble.

3. The calcium oxalate precipitate is allowed to stand 3 hours or longer, though most of the oxalate separates in a much shorter time. Especially in the case of direct estimations in blood serum the 3 hours' standing gives more uniform results than shorter periods. L. and P., *loc. cit.*

4. A reprecipitation of the calcium oxalate is made in order to obtain it free of magnesium (except possibly a trace) and with only a little excess of ammonium oxalate.

5. The dilute HCl solution of the calcium oxalate should be 8 to 10 cc. If a smaller volume were used, some calcium oxalate might separate at a later stage in the procedure.

6. Laidlaw and Payne suggest introducing a *minute* amount of crystalline calcium alizarinate after the 5 drops of strong ammonia have been added to the hot (80°) mixture. This insures a complete and ready separation of the calcium alizarinate in a crystalline form and hence facilitates filtration.

7. In filtering the calcium alizarinate, "care must be taken not to suck much air through the filter since acids, even the carbonic acid of the air, will decompose the calcium salt." L. and P., *loc. cit.*

8. Paper filters are not as satisfactory as asbestos. Ordinary filter paper contains significant amounts of interfering salts.

9. The alizarin solutions of the sample and standard should be prepared under as nearly the same conditions as possible in order

that their tints match. A large excess of ammonia gives a purple tint instead of the red one obtained when the solution is just ammoniacal. A small difference in the ammonia content of sample solution and standard is insignificant, thanks to the buffer effect of the oxalate.

10. Laidlaw and Payne<sup>2</sup> have tried the method on an artificial salt mixture, "made up to imitate the ash of blood serum but with the magnesium in excess." The solution had the following composition:

	Grams
NaCl.....	4.6340
KH <sub>2</sub> PO <sub>4</sub> .....	0.0395
MgSO <sub>4</sub> .....	0.7577
CaCO <sub>3</sub> .....	0.2410
HCl.....	8 cc.
Water to make 500 cc. of solution.	

A trace of iron was also present but was no disadvantage since it may be present in blood serum ash from hemoglobin. Table XI gives results obtained by Laidlaw and Payne when using various amounts of the above solution.

TABLE XI

Solution Taken, Cc.	Ca Found Mg.	Calculated Mg.	Difference Mg.
0.9	0.171	0.1737	-0.0027
0.7	0.140	0.135	+0.005
0.6	0.117	0.115	+0.002
0.5	0.102	0.096	+0.006
0.4	0.0815	0.077	+0.0045
0.3	0.056	0.058	-0.002

The same authors made calcium determinations on mixed samples of human blood serum and found the results practically the same, whether the estimations were made on the ash of the serum or directly without ashing.

11. Experiments by Laidlaw and Payne on heated serum indicate that heating causes part of the calcium to combine with the serum proteins and thereby prevents direct estimation.

<sup>2</sup> *Loc. cit.*

**DETERMINATION OF CALCIUM AS THE OLEATE \***

The method depends upon the pale yellow color produced by colloidal calcium oleate. Magnesium must be absent since it produces a similar color.

**Reagents.**

1. Reagent A. Dissolve 20 grams of Rochelle salt and 7.5 grams of KOH in 100 cc. of water.

2. Reagent B. Dissolve 2 grams of oleic acid and 0.5 gram of KOH in 400 cc. of alcohol and dilute to a liter.

**Procedure.**—A solution of the sample containing 0.005 to 0.01 mg. of calcium is treated with 1 cc. of each of the reagents, diluted to 50 cc., shaken, allowed to stand one hour, and then compared with a series of standards produced similarly.

**DETERMINATION OF CALCIUM IN URINE \***

This is a turbidimetric method. The calcium is first separated from the magnesium as oxalate, according to the method of McCruden.<sup>5</sup> The calcium oxalate is then dissolved in dilute hydrochloric acid, the calcium reprecipitated as calcium soap, and the cloudy suspension compared in a colorimeter with a standard similarly prepared.

**Reagents.**

1. Hydrochloric acid, sp. gr. 1.19.

2. Hydrochloric acid, 1 N.

3. Oxalic acid, 2 per cent.

4. Ammonium hydroxide, sp. gr. 0.90.

5. Ammonium oxalate, 0.5 per cent.

6. Sodium acetate, 10 per cent.

7. Potassium ricinate reagent. Dissolve 15 grams of potassium hydroxide in a mixture of 25 cc. of water and 100 cc. of alcohol. Warm the solution, add 100 cc. of castor oil, shake thoroughly and heat under a reflux condenser on a hot water-bath until a drop or two of the liquid dissolves in water with no free oil showing. About 7 hours' heating are usually required. Keep this as a stock solution. Withdraw 35 cc. of

\* A. Gregoire, E. Carpiaux, E. Larose, and T. Sola, *Bull. soc. chim. Belg.*, **32**, 123 (1923).

\* H. Lyman, *J. Biol. Chem.*, **21**, 551 (1915).

\* J. Biol. Chem., **10**, 187 (1911-12).

the stock solution and dilute it with 965 cc. of distilled water in which have been dissolved 9 grams of sodium hydroxide. The reagent should be clear and almost colorless. It must be made up fresh from the stock solution once a week.

8. Standard calcium oxalate solution. Dissolve 0.5468 gram of calcium oxalate,  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ , in 0.5 N hydrochloric acid, dilute to a liter with the 0.5 N acid, and thoroughly mix. One cubic centimeter of this solution contains 0.15 mg. of calcium.

**Procedure.**—If the urine is alkaline, slightly acidify it, filter, make just alkaline with concentrated ammonium hydroxide and then just acid with concentrated hydrochloric acid. Cool the solution if necessary and add 5 drops of concentrated hydrochloric acid for each 100 cc. of urine.

A distinct turbidity (due to phosphate) formed upon making the urine alkaline may serve as an indicator; otherwise, use litmus paper. The intensity of the turbidity may also serve to indicate the amount of urine to be taken for the analysis. In the case of a heavy turbidity take 5 cc. and for a slight one use 8 cc., 10 cc., or more. Usually with normal adults on a mixed diet 8 cc. gives the best results.

Transfer by means of a pipette 5, 8, or 10 cc. of the acidified urine to a small Erlenmeyer flask, add 1 cc. of 2 per cent oxalic acid solution and 1 cc. of 10 per cent sodium acetate solution. Stopper the flask and thoroughly shake for 10 minutes. Rinse the stopper with a cubic centimeter or two of 0.5 per cent ammonium oxalate solution, pour the mixture into a centrifuge tube, rinse the flask with 2 cc. of the ammonium oxalate solution, and centrifuge until the supernatant liquid is perfectly clear. Two or 3 minutes will suffice. Carefully decant the supernatant liquid so as not to disturb the precipitate, wash the latter with 10 cc. of the ammonium oxalate solution, centrifuge, and carefully decant as before. Dissolve the precipitate in 5 cc. of 1 N hydrochloric acid. Use a stirring rod to hasten solution. Should the precipitate dissolve slowly, place the tube in hot water for several minutes. Cool, and pour the solution into the original Erlenmeyer flask, rinsing both the tube and stirring rod with 5 cc. of distilled water. Stir the solution several minutes to dissolve any precipitate on the walls of the flask. Place in a similar flask 10 cc. of the standard calcium oxalate solution. Then by means of a pipette (with tip broken off) run into each flask 20 cc. of the potassium ricinate reagent. The flasks should be agitated during the addition of the reagent. Thor-

oughly mix the suspensions, let them stand 2 minutes and then match the turbidities in a Duboscq or similar colorimeter.

If 10 cc. of urine were taken for the analysis and the unknown set at 15 mm., the reading of the standard will equal the number of milligrams of calcium in 100 cc. of urine. Deeper clouds may be read at 10 mm., and lighter ones at 20 mm. In any case where the unknown is set at a given height and the standard read against it, the number of milligrams of calcium in 1 cc. is obtained by the following ratio:

$$\frac{\text{Reading of the standard} \times 1.5}{\text{Height at which unknown was set} \times \text{number of cc. of urine taken}}$$

#### Notes.

1. An alkaline urine made slightly acid is filtered in order that the subsequently precipitated calcium oxalate may be easily centrifuged out.

2. Thoroughly shaking the calcium oxalate precipitate for 10 minutes takes the place of standing over night.<sup>6</sup> The precipitation must not be made in a centrifuge tube, since there is a tendency for the oxalate crystals to cling to the walls of the tube.

3. If the amount of urine taken for analysis contains less than 0.75 mg. or more than 2.5 mg. of calcium, the readings will not be quantitative and another determination must be made using a volume of urine which has a calcium content between these limits. The difficulty cannot be overcome by diluting either the unknown or the standard after precipitation, since results thus obtained will not be accurate.

4. If 5 cc. of the urine contain more than 2.5 mg. of calcium, it should be diluted and an aliquot part taken for analysis. On the other hand, if the calcium content is so low that an amount containing 0.75 mg. will not go into the centrifuge tube, the necessary quantity is precipitated (using a proportionately greater volume of sodium acetate solution) and centrifuged in successive portions.

5. Large centrifuge tubes with round bottoms cannot be used, since part of the precipitate will always be lost during the decantation.

6. The clouds produced by precipitating calcium ricinate continue to grow darker for 15 to 20 minutes, but in two solutions of dif-

<sup>6</sup> F. H. McCrudden, *J. Biol. Chem.*, **10**, 187 (1911-12); *cf.* H. Lyman, *ibid.*, **21**, 553 (1915).



ferent strength (calcium content between 0.75 and 2.5 mg.) the increase of turbidity is parallel. Therefore, if the unknown and standard solutions are treated as near the same time as possible, correct readings may be made in 1 or 2 minutes.

7. It is not necessary to remove either the glycerin or the slight excess of alcohol from the ricinate, since experiment has shown that they do not interfere.<sup>7</sup> The excess of sodium hydroxide is added to neutralize the acid in which the calcium oxalate is dissolved and which would otherwise precipitate fatty acids, thus obscuring the soap cloud. Potassium hydroxide does not give good results.

8. Table XII taken from Lyman<sup>8</sup> gives the results of ten analyses of urine by the above method, checked by the method of McCrudden.

TABLE XII

New Method Calcium per 100 cc., Mg.	Method of McCrudden Calcium per 100 cc., Mg.	New Method Calcium per 100 cc., Mg.	Method of McCrudden Calcium per 100 cc., Mg.
16.5	16.6	27.3	27.2
26.4	26.4	19.0	19.1
19.5	19.9	34.2	34.1
35.0	34.9	15.1	14.9
11.6	12.2	30.1	29.9

#### DETERMINATION OF CALCIUM IN FECES<sup>9</sup>

This determination is made exactly as directed for the determination of calcium in urine except that with feces a little more preparation of the substance is required. After thorough mixing, 5 or 6 grams of the feces are accurately weighed, mixed with a little concentrated sulfuric acid, and burned to a white powder in a small silica dish. Heat gently at first, on account of the considerable frothing. Wash the residue into a 100 cc. volumetric flask with 50 cc. of 2 N hydrochloric acid, make up to the mark, shake thoroughly, and filter. Dilute 50 cc. of the filtrate to 100 cc. with distilled water and proceed with this solu-

<sup>7</sup> H. Lyman, J. Biol. Chem., **21**, 555 (1915).

<sup>8</sup> J. Biol. Chem., **10**, 556 (1911-12).

<sup>9</sup> H. Lyman, J. Biol. Chem., **21**, 555 (1915).

tion exactly as in the case of urine (page 144), except that 2 cc. of sodium acetate are used instead of 1 cc.

**Notes.**

1. Without the excess of acetate the precipitate is finely divided and difficult to handle.
2. In case the calcium content is far from normal, the volume up to which the ash solution is made can be varied at will.

## CHAPTER XII

### CARBON AND CYANIDE

#### DETERMINATION OF CARBON IN STEEL

WHEN a steel is dissolved in nitric acid a brown-colored solution is obtained, the intensity of the color being proportional to the amount of carbon present. This coloration is said to be due to some organic compound which at first is seen as a precipitate but later dissolves upon heating.<sup>1</sup> Only the carbon present as carbide is transformed into the brown color. Any carbon present as graphite is not acted upon by the acid and, hence, results by the colorimetric method will be low. High carbon steels are apt to contain much graphite but this is easily detected by the insoluble black residue remaining after the acid treatment. The carbon in such steels must be determined by the combustion method and it is advisable to check the colorimetric determinations at frequent intervals by combustion. In a series of ten or more steels every fifth sample should be checked by the combustion method. If the two methods agree within 0.01 to 0.03 per cent for 0.4 to 1.0 per cent carbon steels, it may be fairly safely concluded that the colorimetric determinations are satisfactory.

There are many possible sources of error (see Notes below) in the colorimetric method for the determination of carbon in steel. The successful use of the method is only gained by long experience and unless the beginner works under the guidance of an experienced operator, or makes frequent checks by the combustion method, his results are apt to be highly inaccurate.

#### Reagents.

1. Nitric acid, sp. gr. 1.20.
2. Standard steels. A series of steels of accurately known carbon content must be available. These steels must be the same kind as the samples, i.e., Bessemer, acid open hearth, basic open hearth, etc., and

<sup>1</sup> Levy, Analyst, **37**, 392 (1912).

should have been drilled under the same conditions as those used in obtaining the sample. The carbon content must be over the range of that in the samples.

**Obtaining the Sample.**—The sample may be taken from the raw cast steel and allowed to cool slowly from the molten state without quenching. Or, if the sample is first cooled to a black heat in a dark room it may then be quenched. Better still is for the operator to anneal the steel so as to avoid the temperature most favorable to the formation of graphite. The steel may then be tested to see whether or not the annealing is perfect. Drill all samples to a uniform thickness. All drillings that are rusty or blued must be rejected.<sup>2</sup>

The carbon content of the standard should be within 10 per cent of that in the sample, the nearer the better—especially if the steels are unannealed.

**Procedure.**<sup>3</sup>—Place 0.100 gram of the sample in a test tube (152 mm. long and 15 to 16 mm. diameter), add 4 cc. of nitric acid, sp. gr. 1.20, and set the tube in a hot water-bath fitted with a rack especially designed for this work. The rack should have a false bottom perforated with many small holes and should be arranged so that each tube is immersed to a depth of 28 mm. (about the level of the liquid in the tube). In no case must the top of the liquid in the tube be below the level of the boiling water, otherwise iron oxide will deposit on the sides of the tube and will later form a brown basic nitrate when dissolved. The latter will cause a variation in the color of the solution. Some laboratories have a practice of placing a glass marble on the top of each tube to reduce the loss of acid due to evaporation. Solution of the carbon is usually complete within 40 minutes.

To complete the solution in from 4 to 7 minutes, the tubes are placed in a sand- or graphite-bath heated to about 190° C. Not more than six tubes should be heated at one time on account of possible wide variation in the temperature in different parts of such a bath, and the tubes should be placed close together. Johnson recommends covering the cluster of tubes with a beaker to prevent too rapid loss of acid.

As soon as all the brown flakes have dissolved, the tubes are removed from the bath and cooled at once in running water. Each solu-

<sup>2</sup> See Chapter XVII (The Annealing of Steel) in Johnson's "Chemical Analysis of Special Steels, Steel-Making Alloys and Graphites," 3d ed., John Wiley & Sons, New York, 1920.

<sup>3</sup> Cf. Johnson, *loc. cit.*, p. 306.

tion is then put in a bent-end comparison tube of 14 cc. capacity and graduated to tenths of a cubic centimeter. The bent-end permits thorough mixing of the solution by a rocking motion of the tube. (See Notes for a more detailed description of the tubes.) The comparison is made by diluting the solution until its color is just faintly lighter than that of the standard, and then deducting 0.01 per cent to allow for overstepping the end-point. The dilution must be made very carefully, the water being added 0.1 to 0.2 cc. at a time and the contents of the tube thoroughly mixed after each dilution. Suppose a 0.60 per cent carbon steel has been taken as the standard and its solution made up to exactly 6 cc. Then if the sample solution is just faintly lighter than the standard when diluted to 6.5 cc., the per cent of carbon is 0.65 less 0.01, i.e., 0.64 per cent. Or, suppose a 0.40 per cent carbon steel is taken as a standard and its solution diluted to exactly 8 cc. Then if the sample solution matches the standard at a dilution of 9 cc., its carbon content is  $0.90 \div 2$ , or, 0.45 per cent.

The solutions may be compared in a color camera, but Johnson recommends holding the tubes on white paper in diffused sunlight or the light from a 50-candle power frosted electric lamp of filament type. The tubes should be held at an angle of  $45^\circ$  to the paper and with their ends touching it. If the tubes are turned so that their graduations touch each other, a clear field is obtained for the comparison.

It is a good practice in making the final comparison to lose track of which is the standard and which is the sample by interchanging the tubes several times, then selecting the lighter (or darker) solution. Repeat this procedure until the same conclusion is reached three times in succession.

Johnson<sup>4</sup> says "a man with a good eye for color and plenty of practice can be counted on not to introduce an error due to the eye of over 0.02 per cent in higher carbons and not over 0.010 per cent in lower carbons, around 0.08 and perhaps not over 0.005 per cent in the latter range."

#### Notes.

1. Johnson recommends using a set of three tubes meeting the following specifications: Capacity 14 cc., graduated to 0.1 cc., outside diameter 12 mm., length of graduated portion 181 mm., followed by 45 mm. of ungraduated tube, and then a bent portion of the tube about

<sup>4</sup> *Loc. cit.*, p. 309.

50 mm. long. The tubes should be of carefully selected tubing, free from scratches and from fine black lines due to minute air bubbles. The three tubes should have the same bore and should be uniform throughout. Hence, when carefully graduated the markings on the three tubes will coincide. The figures and graduation lines should be very small so as not to interfere with the field of vision.

2. All determinations should be run in duplicate and the average taken. No advantage is gained by working with a sample larger than 0.100 gram.

3. The color of the nitric acid solution of a carbon steel will vary with the heat treatment the steel has undergone. This may introduce an error of 10 to 20 per cent of the actual carbon content. A perfectly annealed steel gives the greatest depth of color for a given percentage of carbon.

4. Unless the drillings of sample and standard are approximately uniform in size, an error of as much as 10 per cent may result. Large, bulky, thick drillings give low results.

5. The presence of graphitic carbon causes low results. The error may vary from 5 to 90 per cent. Fortunately, the presence of much graphitic carbon can easily be detected by a black residue remaining after solution of the steel, so that an error of over 5 per cent is not likely to be introduced.

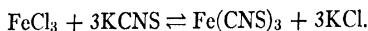
6. Manganese lightens the color of the solution but may be disregarded if not over 1 per cent is present. Nickel has a similar effect. A high nickel content will give a greenish colored solution difficult to match. The presence of over 1 per cent of silica also gives a greenish tinge to the solution. Copper, chromium, and cobalt also interfere. When the sample is known to contain any one or more of the above substances, a standard containing the same amounts of such substances should be employed. From the above it follows that the composition of both sample and standard should be accurately known.

#### DETERMINATION OF HYDROGEN CYANIDE BY FORMATION OF PRUSSIAN BLUE

This method<sup>5</sup> is especially adapted to the estimation of small quantities of hydrocyanic acid in cyanogenetic plants. The usual presence of reducing agents in plant distillates excludes the various titration methods as well as the silver gravimetric methods and the

<sup>5</sup> A. Viehoveer and C. O. Johns, J. Am. Chem. Soc., **37**, 601 (1916).

picric acid colorimetric method. Moreover, the quantities to be determined are often too small to permit the use of volumetric or gravimetric methods. The objection to the thiocyanate colorimetric method is based on the nature of the reaction between a thiocyanate and ferric chloride:



The equilibrium of this reaction is so easily disturbed by the addition of acids, ferric chloride, or other salts, that it is difficult to adjust conditions so as to obtain constant results. The Prussian blue method of Viehoveer and Johns is satisfactory with less than 0.5 mg. of hydrocyanic acid and will detect less than 0.01 mg. of the acid.

#### Reagents.

1. Nitric acid, 30 per cent.
2. Sodium hydroxide, 6 N.
3. Ferrous sulfate, 3 per cent. Freshly prepared.
4. Potassium fluoride. Solid.
5. Standard potassium cyanide. Dissolve 0.5000 gram of potassium cyanide, dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 0.5 mg. of KCN, equivalent to 0.21 mg. of HCN.

**Procedure.**—A measured quantity of the sample solution is placed in a 200 cc. round-bottom flask and made slightly alkaline with sodium hydroxide. The flask is then connected to a condenser (by means of an adapter) and vacuum pump, and the liquid concentrated under reduced pressure. The heat is supplied by immersing the flask in a water-bath at 60° to 70°. Concentrate the liquid to less than 1 cc. and then add 0.5 cc. of 3 per cent ferrous sulfate solution and about 0.05 gram of potassium fluoride. Exhaust the flask at once by means of a water suction pump. Rotate the flask so as to mix its contents. After evacuating for about 10 minutes detach the flask and acidify the mixture by adding 30 per cent nitric acid drop by drop. The blue color appears at once, except in case only a minute quantity of hydrocyanic acid is present. If the color does not appear at once, warm the solution in a water-bath to about 50°. Dilute the suspension to a volume that gives a color intensity suitable for comparison with a suspension of Prussian blue made from a known weight of potassium cyanide. A Prussian blue suspension made from 1 mg. of potassium cyanide and

diluted to 25 cc. gives a color of convenient intensity. A Duboscq or similar type colorimeter is recommended.

#### Notes.

1. The liquid is concentrated under diminished pressure and below  $70^{\circ}$  to insure against loss of hydrocyanic acid (b. p.  $85^{\circ}$ ).

2. The maximum color is obtained only when the volume of the solution to be tested is not over 1.5 cc. If more than 1 mg. of potassium cyanide is present the volume of the test solution may be increased a little without loss.

3. The presence of certain salts in the liquid, especially potassium fluoride, has proved to be of great advantage. "On acidifying in the final stage of the test the color appears at once and is very brilliant. The absence of a green shade makes it particularly suitable for comparison with a standard. If the acid is added very gradually the iron hydroxides dissolve and a colorless liquid with a white precipitate is obtained. On the addition of more acid the blue color appears. This is explained<sup>6</sup> by the fact that ferric salts produce a complex salt,  $K_3FeF_6$ , with potassium fluoride. When an excess of acid is added this complex salt is decomposed and the ferric ions needed for the formation of Prussian blue are furnished."<sup>7</sup>

4. It is not necessary to add ferric salt since enough of the ferrous salt is oxidized during the course of the procedure to furnish the required ferric ions. An excess of ferric salt is to be avoided. Hence, the air is removed from the solution by means of a water vacuum pump so as to prevent the oxidation of too much of the ferrous hydroxide to ferric.

5. If the cyanide solution is sufficiently concentrated that evaporation is unnecessary, the test is made in a test tube, air being kept out by means of a stopper and the tube rotated only enough to mix the reagents. Let the mixture stand about 10 minutes and then acidify.

6. The quantities of reagents specified in the procedure are sufficient for 1 to 2 mg. of potassium cyanide. If less than 1 mg. of cyanide is present, reduce the quantities of the reagents accordingly. A large excess of reagents must be avoided in order to obtain the maximum intensity of color.

<sup>6</sup> Greef, Ber., **40**, 2511 (1913).

<sup>7</sup> Viehhoever and Johns, *loc. cit.*



7. "When the analysis is carried out as described the maximum error should not exceed more than 1 part in 20. Thus in a plant giving 20 mg. of potassium cyanide per 100 grams of plant the results might vary by 0.001 per cent if 100 grams of material are used for analysis."<sup>8</sup>

**DETERMINATION OF CYANIDE BY CONVERSION INTO THIOCYANATE AND FORMATION OF FERRIC THIOCYANATE<sup>9</sup>**

The following method is based upon the red color of ferric thiocyanate. The cyanide is first converted into thiocyanate and the latter converted into ferric thiocyanate by the addition of ferric chloride solution. The method was originally developed to estimate the cyanide content of certain poisonous plants, but is applicable to any substance containing 1 per cent of cyanide or less.

**Reagents.**

1. Hydrochloric acid, 6 N.
2. Sulfuric acid, sp. gr. 1.84.
3. Potassium hydroxide, 4 per cent.
4. Ammonium polysulfide, 6 N. Place 200 cc. of 15 N  $\text{NH}_4\text{OH}$  in a flask immersed in running water or iced water and saturate with  $\text{H}_2\text{S}$ . Then add 200 cc. of 15 N  $\text{NH}_4\text{OH}$ , dilute with water to 1000 cc., add 25 grams of flowers of sulfur, digest for several hours or over night, and filter.
5. Ferric chloride, 0.5 per cent.
6. Standard thiocyanate solution. Dissolve 15 grams of pure potassium thiocyanate in a liter of water. Standardize gravimetrically with silver nitrate and dilute so that 1 cc. contains 14.93 mg. of KCNS, which is equivalent to 10 mg. of KCN. One hundred cubic centimeters of this solution are diluted to a liter and thoroughly mixed. One cubic centimeter of the diluted solution contains 1.49 mg. of KCNS, which is equivalent to 1 mg. of KCN.

**Procedure.**—If the substance is an inorganic liquid, a measured volume of it is placed directly in a Nessler tube. If an inorganic solid, the substance must be dissolved in a faintly alkaline solution to prevent loss of hydrocyanic acid and made faintly acid just before adding the ferric chloride. In case the sample is an organic solid, a weighed

<sup>8</sup> Viehovever and Johns, *loc. cit.*

<sup>9</sup> M. O. Johnson, J. Am. Chem. Soc., **38**, 1230 (1916); a modification of the method of C. K. Francis and W. B. Connell, J. Am. Chem. Soc., **35**, 1624 (1913).

quantity (50 grams if the cyanide content is only a few hundredths of a per cent) of the finely ground substance is triturated with 100 cc. of water and washed into a liter distilling flask with 100 cc. of water. Connect the flask to a condenser whose delivery end dips into 50 cc. of a 4 per cent potassium hydroxide solution. Now carefully add 50 cc. of concentrated sulfuric acid and connect the flask at once to the condenser. Distill over about 150 cc. of the liquid, taking care that the delivery tube always dips under the potassium hydroxide solution so as to prevent loss of hydrocyanic acid. Transfer the caustic potash solution of the distillate to a 250 cc. volumetric flask, dilute to the mark, mix thoroughly, place 50 cc. of the solution (containing 0.1 to 8 mg. KCN) in a small porcelain casserole, and add to it 1 cc. of yellow ammonium sulfide (or 5 cc. of a 4 per cent solution of potassium sulfide). Evaporate to dryness on a water-bath, take up the residue with 10 cc. of acetone and rub with a small glass or porcelain pestle to effect better extraction. Decant the acetone into a small evaporating dish and repeat the extraction twice. Combine the acetone extracts and evaporate to dryness on the water-bath. Remove the dish and allow it to cool to room temperature. Take up the residue in water, dilute to 50 cc. in a Nessler tube, add 2 cc. of 0.5 per cent ferric chloride solution, and gently mix. Match the color at once against a standard ferric thiocyanate solution prepared at the same time the thiocyanate is added to the sample solution. Any of the usual methods of comparison may be employed, but the methods of balancing and duplication are best.

For the balancing method, add 2 cc. of ferric chloride solution to 25 cc. of the standard thiocyanate solution (containing 1.49 mg. KCNS per cubic centimeter), dilute to 250 cc. and mix. One cubic centimeter contains 0.149 mg. of KCNS and is equivalent to 0.1 mg. of KCN.

To match by the method of duplication, add the standard thio-sulfate solution (containing 1.49 mg. of KCNS per cubic centimeter) drop by drop, with gentle stirring, to a blank of 40 cc. of water containing 2 cc. of ferric chloride solution, finally diluting to the 50 cc. mark with water when the color of the sample and standard is the same.

#### Notes.

1. A permanent series of standards cannot be obtained on account of the fairly rapid fading of ferric thiocyanate due to the reduction of

the iron to the ferrous state by other substances than normal thiocyanic acid. Chief among these substances is isodithiocyanic acid, which is always formed when thiocyanates are acidified. Hydrocyanic acid and hydrogen sulfide are decomposition products of thiocyanic acid and it is possible that they too take part in the reduction of the ferric salt. The color fading is also probably aided by the action of light and, hence, the thiocyanate solutions should be protected from direct sunlight. See the thiocyanate method (Stokes and Cain) for the colorimetric determination of iron, page 218.

The ferric chloride should be added to sample and standard at the same time and comparison made as soon as possible thereafter.

2. Fluorides, phosphates, arsenates, iodates, oxalates, tartrates, and citrates interfere markedly with the analysis, and acetates and sulfates to a lesser degree.<sup>10</sup> Chlorides, bromides, and iodides do not interfere.<sup>11</sup>

3. If the distillate is colored by organic matter and also the acetone extract, the following procedure should be used:

After evaporating the combined acetone extracts to dryness, take up the residue in 15 cc. of water and pour into a small separatory funnel. Wash the dish with two 5 cc. portions of water, pouring the wash water into the funnel. Add to the funnel 25 cc. of ethyl acetate which has previously been extracted several times with water to remove any soluble impurities. Shake well and allow to settle. The yellow color will be taken up by the ethyl acetate. Drain off the clear water solution into an evaporating dish and evaporate to dryness. Take up in water, make up to 50 cc. in a Nessler tube and compare with standard as usual.

<sup>10</sup> H. N. Stokes and J. R. Cain, *J. Am. Chem. Soc.*, **29**, 409 (1907).

<sup>11</sup> C. K. Francis and W. B. Connell, *J. Am. Chem. Soc.*, **35**, 1627 (1913).

## CHAPTER XIII

### CHLORINE, CHLORATE, AND PERCHLORATE

#### DETERMINATION OF FREE CHLORINE BY ORTHO-TOLIDINE

THIS method depends upon the yellow to orange color produced by the action of small amounts of free chlorine on acid solutions of ortho-tolidine. It is especially adapted to water analysis but may be used for the estimation of free chlorine in other solutions.

#### Reagents.

1. Ortho-tolidine. Dissolve 1 gram of *o*-tolidine in a liter of dilute hydrochloric acid (100 cc. conc. acid diluted to 1000 cc.). The *o*-tolidine should melt at 129° C. It may be obtained from the Eastman Kodak Co., Rochester, N. Y., or may be prepared by extracting the technical product with water in a Soxhlet apparatus.

2. Copper sulfate. 1.5 grams copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , and 1 cc. of sulfuric acid, sp. gr. 1.84, are dissolved in distilled water and made up to 100 cc.

3. Potassium bichromate. (A) 0.025 gram of potassium bichromate and 0.1 cc. of sulfuric acid, sp. gr. 1.84, are dissolved in distilled water and made up to 100 cc.

(B) 0.25 gram of potassium bichromate and 1 cc. of sulfuric acid, sp. gr. 1.84, are dissolved in distilled water and made up to 100 cc.

4. Preparation of permanent standards. See Table XIII.

**Procedure.**—Place 100 cc. of the sample in a Nessler cylinder, add 1 cc. of *o*-tolidine reagent, mix, let stand for 5 minutes, and then match the color against a series of copper-bichromate standards. If the chlorine content is greater than 3 parts per million, use more of the *o*-tolidine reagent.

#### Notes.

1. The *o*-tolidine method is delicate enough to detect 0.005 part of free chlorine per million parts of water.

TABLE XIII

Chlorine, parts per million	CuSO <sub>4</sub> , cc.	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> , cc. (Solution A)
0.01	.....	0.8
0.02	.....	2.1
0.03	.....	3.2
0.04	.....	4.3
0.05	0.4	5.5
0.06	0.8	6.6
0.07	1.2	7.5
0.08	1.5	8.7
0.09	1.7	9.0
0.10	1.8	10.0
(Solution B)		
0.10	1.8	1.0
0.20	1.9	2.0
0.30	1.9	3.0
0.40	2.0	3.8
0.50	2.0	4.5
0.60	2.0	5.1
0.70	2.0	5.8
0.80	2.0	6.3
0.90	2.0	6.7
1.00	2.0	7.2
2.00	2.0	12.0
3.00	2.0	21.0
4.00	2.0	30.0
5.00	2.0	39.0
6.00	2.0	46.0
7.00	2.0	56.0
8.00	2.0	63.0
9.00	2.0	70.0
10.00	2.0	75.0

2. The *o*-tolidine is dissolved in a dilute hydrochloric acid solution in order to insure a definite color change. An acetic acid solution of *o*-tolidine does not give reliable results probably due to the effect of varying degrees of alkalinity in different waters.

3. The color obtained by the action of free chlorine on *o*-tolidine is probably due to the oxidation of the latter. The following oxidizing reagents have been shown by L. P. Kinnicutt<sup>1</sup> to produce color reactions with *o*-tolidine: ozone, nascent oxygen, sodium nitrite, ammo-

<sup>1</sup> Cf. J. W. Ellms and S. J. Hauser, J. Ind. Eng. Chem., **6**, 554 (1914).

nium persulfate, ferric chloride, ferric alum, potassium permanganate, potassium bichromate, sodium peroxide, lead peroxide, hydrogen peroxide, bromine, iodine, and nitric acid. With the exception of ferric salts and nitrite, there is only slight probability of natural waters (or even sewage) containing any of the oxidizing agents mentioned.

One part of iron per million parts of water will produce a color with the *o*-tolidine reagent corresponding to 0.01 part of chlorine per million. This amount of iron is not usually found in surface waters, but may be present in ground waters.

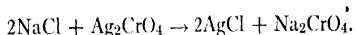
0.09 part of nitrogen (as nitrite) per million parts of water produces a color with the *o*-tolidine reagent corresponding to 0.01 part of chlorine. Such a high nitrite content is not likely to be found in surface waters, unless they are badly polluted. Sewage and especially sewage effluents may contain nitrites in amounts sufficient to interfere with the accuracy of the analysis.

#### REFERENCES

1. J. W. Ellms and S. J. Hauser, *J. Ind. Eng. Chem.*, **5**, 915, 1030 (1913).
2. W. H. Dittoe and L. H. Van Buskirk, *Ohio State Board of Health, Bull.* **3**, No. 1 (January, 1913).

#### DETERMINATION OF CHLORIDES IN BLOOD

This method is based upon a difference in solubilities of silver chloride and red silver chromate, the former being only about one-thirtieth as soluble as the latter. From this it follows that silver chromate will dissolve in a solution of chlorides, the silver reprecipitating as silver chloride and an equivalent amount of chromate going into solution. The reaction is represented by the following equation:



By matching the yellow color of the solution against a standard chromate solution the amount of chromate in the sample is determined and from this the amount of chloride present may be calculated.

The analysis is made with a protein-free blood filtrate, using tungstic acid as a protein precipitant.

**Reagents.**

1. Sulfuric acid,  $\frac{2}{3}$  N.
2. Ammonium hydroxide, 2 per cent.
3. Sodium tungstate,  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ , 2 per cent.
4. Potassium oxalate.
5. Silver chromate. (Red modification.) Two hundred cubic centimeters of a 5.5 per cent solution of potassium chromate are added slowly to 100 cc. of a boiling solution of 10 per cent silver nitrate. A precipitate of silver chromate settles out rapidly. Add the chromate solution drop by drop until a slight excess has been added as indicated by the pale yellow solution produced. Cool, filter through a Buchner funnel, wash thoroughly and allow to air-dry.
6. Magnesium carbonate.
7. Standard potassium chromate solution. Make a solution of pure potassium chromate containing 0.4 gram of the salt per liter. Standardize against a 0.02 N solution of sodium chloride.

**Procedure.**—"Transfer a measured amount of blood into a flask having a capacity of fifteen to twenty times that of the volume taken. Dilute the blood with 7 volumes of water and mix. With an appropriate pipette add 1 volume of 10 per cent solution of sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ) and mix. With another suitable pipette add to the contents in the flask (with shaking) 1 volume of  $\frac{2}{3}$  normal sulfuric acid. Close the mouth of the flask with a rubber stopper and give a few vigorous shakes. If the conditions are right hardly a single air bubble will form as a result of the shaking. Much oxalate or citrate interferes with the coagulation and later with the uric acid determination. Twenty milligrams of potassium oxalate is ample for 10 cc. of blood. Citrate, except in the minimum amount, is to be avoided. When a blood is properly coagulated, the color of the coagulum gradually changes from pink to dark brown. If this change does not occur, the coagulation is incomplete, due, in every case we have encountered, to too much oxalate or citrate. In such an emergency the sample may be saved by adding 2 normal sulfuric acid drop by drop, shaking vigorously after each addition and allowing the mixture to stand for a few minutes before adding more, until the coagulation is complete. Pour the mixture on a filter large enough to hold the entire contents of the flask and cover with a watch-glass. If the filtration is begun by pouring the first few cubic centimeters of the mixture down

the double portion of the filter paper and withholding the remainder till the whole filter has been wet, the filtrates are almost invariably as clear as water from the first drop. If a filtrate is not perfectly clear, the first 2 or 3 cc. may have to be returned to the funnel."<sup>2</sup>

"10 cc. of the Folin and Wu filtrate are pipetted into a small conical centrifuge tube (which has been previously cleaned with warm chromic acid solution). A pinch of magnesium carbonate is added to insure neutrality of the liquid. The contents of the tube are stirred with a thin glass rod. A small quantity (about 0.05 g.) of silver chromate is introduced and thoroughly stirred into the solution. If all the red particles disappear more chromate must be added. After washing off the stirring rod into the tube, the tube is centrifuged for 2 minutes. (See Note 7.) The contents are then decanted through a small filter, into a 25 cc. volumetric flask, great care being taken not to disturb the residue at the bottom of the tube. After the addition of 10 cc. of water to the tube, the centrifuging is repeated for 5 minutes. (See Note 7.) The contents of the tube are then filtered into the volumetric flask. The solution has a slight turbidity which is cleared up by the addition of 1 cc. of a 2 per cent ammonium hydroxide solution. Enough water is added to bring the solution to the mark. After mixing, comparison is made with a standard potassium chromate solution containing 0.4 gram of the salt per liter."<sup>3</sup>

#### Notes.

1. Michaelis<sup>4</sup> suggests that the colors be matched through a blue glass on account of the difficulty in comparing yellows.

2. "Silver phosphate is slightly less soluble than silver chromate and it would be expected that silver chromate would dissolve in a solution containing phosphate. This does take place, but in very dilute solutions such as the blood filtrate the color develops very slowly. Furthermore, the phosphates of the blood are probably acid phosphates which do not react with the chromate. This was shown by adding 0.0010 gram of monosodium hydrogen phosphate to 5 cc. of blood filtrate, an amount which would correspond to an extreme case of phosphate retention. As a check, 5 cc. of the same filtrate were taken and both filtrates were treated with magnesium carbonate and silver

<sup>2</sup> O. Folin and H. Wu, *J. Biol. Chem.*, **38**, 84 (1919).

<sup>3</sup> M. L. Isaacs, *J. Biol. Chem.*, **53**, 17 (1922).

<sup>4</sup> Deutsch, *med. Woch.*, **47**, 465 (1921).



chromate. No difference of color could be observed. Other silver salts which are less soluble than the chromate are either absent or present in negligible quantities in the filtrate."<sup>5</sup>

3. The "suitable pipette" referred to in the procedure is simply a pipette with a long tip and graduated into 1 cc. portions from the tip. It has a capacity of 15 cc. The advantage of such a pipette is that practically the whole sample of blood may be used, since 7, 9, 12, etc., cc. may be taken just as easily as 5 or 10 cc.

4. The concentration of the sodium tungstate and sulfuric acid must be correct. The whole of the tungstic acid is liberated by the sulfuric acid and there should be about a 10 per cent excess of the latter to neutralize the carbonate usually present in commercial sodium tungstate. A greater excess of sulfuric acid is to be avoided, otherwise much of the uric acid will be lost. The blood filtrate should be neutral or just faintly acid to Congo red paper.

5. If the blood filtrate is to be kept longer than 2 or 3 days, 1 or 2 drops of toluene or xylene should be added to the filtrate from each 10 cc. of blood. Xylene seems to be as good a preservative as toluene.

6. The tungstic acid method for the precipitation of blood proteins works equally well with any kind of blood tried by Folin and Wu<sup>6</sup>—human, beef, sheep, chicken, dog, and rabbit.

7. *Much time and effort may be saved by filtering off the excess silver chromate instead of centrifuging.*<sup>7</sup> A good quality filter paper, suitable for retaining finely divided precipitates, must be used. In order to avoid using too much wash-water, the diameter of the filter paper should be about 4 cm.

8. Dupray<sup>8</sup> has published a modification of Isaacs' which has the advantage of a more easily-read color. In this modified method, also, it would be a distinct advantage to filter as directed in the preceding note. For another modification along similar lines, see Yoshimatsu, Tōhoku J. Exptl. Med. **7**, 553 (1926). (The original article is in English.)

#### DETERMINATION OF CHLORATES BY ANILINE CHLORIDE

This method is based upon the blue coloration produced by adding a solution of aniline chloride in hydrochloric acid to a solution con-

<sup>5</sup> Isaacs, *loc. cit.*

<sup>6</sup> *Loc. cit.*

<sup>7</sup> Private communication from Dr. M. L. Isaacs.

<sup>8</sup> J. Biol. Chem., **58**, 675 (1923-24).

taining chlorate. Certain other oxidizing agents must be absent. (See Note 2.)

### Reagents.

1. Solution A. Prepare a colorless solution of 50 grams of pure aniline chloride in one liter of hydrochloric acid of such strength that the resulting solution has a sp. gr. of 1.20.

2. Solution B. Same as Solution A, except that the hydrochloric acid strength is adjusted so that the resulting aniline chloride solution has a sp. gr. of 1.145.

3. Standard potassium chlorate solution. Prepare a solution containing 1 mg. of  $\text{ClO}_3$  per cubic centimeter, using sodium or potassium chlorate.

**Procedure.**—Use a 5 cc. portion of the sample, diluting or concentrating if necessary. If the sample solution contains between 0.5 and 7 mg. of chlorate per 5 cc., add 20 cc. of solution A; if it contains between 0.1 and 2 mg. in the 5 cc., add 20 cc. of solution B. A violet color develops immediately but changes in a few minutes to blue. Allow the solution to stand 25 minutes in case solution A was used and 15 minutes if solution B was used. Then compare against a series of standards prepared along with the sample.

### Notes.

1. The aniline chloride reagent<sup>9</sup> will detect as little as 0.007 mg. of  $\text{KClO}_3$ .

2. Chlorine, hypobromites, hydrogen peroxide, manganese dioxide, chromates, etc., also produce a violet to blue color with the aniline chloride reagent and, hence, must be absent. Strong reducing agents, as well as any substance that produces a color reaction, also interfere. Carbon, sulfur, phosphates, perchlorates, ferric chloride, nitrates, and small amounts of nitrites do not interfere with the test.

### DETERMINATION OF PERCHLORATES BY NITROSODIMETHYLANILINE<sup>10</sup>

This method depends upon the violet color obtained by the reaction between perchlorates and nitrosodimethylaniline and is especially adapted to the estimation of perchlorates in Chile saltpeter.

<sup>9</sup> J. F. Virgili, *Ann. chim. anal. appl.*, **14**, 85 (1909).

<sup>10</sup> A. Monnier, *Arch. sci. phys. nat.*, **42**, 210 (1916).

**Reagents.**

1. Nitrosodimethylaniline solution. Dissolve 1 gram of nitrosodimethylaniline in alcohol and dilute to a liter.
2. Standard perchlorate solution. Dissolve 0.1393 gram of potassium perchlorate in water and dilute to a liter. Mix thoroughly and dilute 10 cc. of the solution to 100 cc. One cubic centimeter of the diluted solution contains 0.01 mg. of  $\text{ClO}_4$ .

**Procedure.**—Dissolve the sample (usually 1 gram is satisfactory) in 25 cc. of water, add 2 cc. of the nitrosodimethylaniline solution, mix, and allow to stand several hours in a Nessler tube. At the same time have ready a series of Nessler tubes containing measured quantities of the standard perchlorate solution diluted to 25 cc., add 2 cc. of the nitrosodimethylaniline solution to each tube, and mix thoroughly. After sample and standards have stood several hours, the color comparison is made.

**Notes.**

1. The nitrosodimethylaniline solution is added to the sample and series of standards at the same time, and allowed to stand several hours so as to develop the full intensity of color.
2. Interfering iodides may be removed with  $\text{Ag}_2\text{O}$ ; iodates and periodates have no effect upon the reagent.

## CHAPTER XIV

### CHROMIUM

#### DETERMINATION OF CHROMIUM BY DISODIUM 1, 8-DIHYDROXYNAPHTHALENE-3, 6-DISULFONATE

THE method depends upon the pink to cherry-red color produced by the addition of König's reagent (see below) to a solution containing chromium in the presence of sulfuric and phosphoric acids. It is well adapted for the determination of chromium in steels. As little as 0.0008 mg. of chromium can be detected.

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Dilute sulfuric acid, 6 N.
3. Nitric acid, sp. gr. 1.42.
4. Phosphoric acid, 85 per cent.
5. Sodium hydroxide, 10 per cent.
6. Sodium peroxide.
7. Disodium 1, 8-dihydroxynaphthalene-3, 6-disulfonate (König's reagent). Use a 1 per cent solution.
8. Standard chromium solution. This solution is prepared by treating a weighed portion of a steel of known chromium content in the same way as that given in the Procedure for the sample under investigation. A Cr-free steel may be used with a known quantity of chromium added.

**Procedure.**—For steels having a chromium content between 0.01 and 0.1 per cent, take a 0.4 gram sample, and for 0.1 to 0.2 per cent chromium, take 0.2 gram. Dissolve the sample in 10 cc. of sulfuric acid, add 0.5 cc. of conc. nitric acid and heat till dense fumes are given off. Cool the solution, dilute with a few cubic centimeters of water, add 50 cc. of 10 per cent sodium hydroxide solution and 1 gram of sodium peroxide. Boil for 5 minutes or until the excess of peroxide is destroyed, cool to room temperature, transfer to a 200 cc. volumetric flask, dilute to the mark and thoroughly mix. Filter off 100 cc. of the

solution, add 2 cc. of 85 per cent phosphoric acid, 8 cc. of concentrated sulfuric acid, 2 cc. of a 1 per cent solution of disodium 1, 8-dihydroxynaphthalene-3, 6-disulfonate, and mix thoroughly. After standing 15 minutes, compare with a standard prepared in the same way. The dilution method must be used. (See Note 2.)

#### Notes.

1. Vanadium produces a brown color, when present in considerable quantities, and will be likely to obscure the result. If the vanadium and chromium contents are about the same, a correction may be made by introducing a similar amount of vanadium in the standard. When the ratio of vanadium to chromium is small the error may be disregarded, except where extreme accuracy is desired. In the latter case, add the same amount of vanadium to the standard as there is in the sample.

2. On account of the peculiar character of the color developed, and the danger of interference, the comparison must be made by the method of dilution.

#### REFERENCES

1. F. Garratt, *J. Ind. Eng. Chem.*, **5**, 298 (1913).
2. P. N. Van Eck, *Chem. Weekblad*, **12**, 6 (1915).
3. A. I. Applebaum, *Chem. Analyst*, **27**, 7 (1918).

#### DETERMINATION OF CHROMIUM BY THE DIPHENYLSEMICARBAZIDE REACTION

The observation of Cazeneuve<sup>1</sup> that diphenylsemicarbazide and chromic acid give a purple-colored solution has been applied to the colorimetric estimation of chromium. The chromium is first converted into chromate by oxidation with sodium peroxide and finally neutralized with acetic acid.

#### Reagents.

1. Sulfuric acid, 6 N. Pour one volume of 95 per cent  $\text{H}_2\text{SO}_4$  into 5 volumes of water.
2. Nitric acid, sp. gr. 1.42.
3. Sodium hydroxide, 2.5 N. Dissolve 100 grams of NaOH in water and dilute to a liter.

<sup>1</sup> *Analyst*, **25**, 331 (1900).

4. Sodium peroxide.

5. Diphenylsemicarbazide reagent. Dissolve 1 gram of diphenylsemicarbazide in 10 cc. of acetic acid and dilute to a liter with water.

6. Standard chromate solution. Dissolve 0.2555 gram of pure potassium chromate,  $K_2CrO_4$ , in water, dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of  $Cr_2O_3$ .

**Procedure.**—Use a sample of 0.4 gram for chromium content between 0.01 and 0.1 per cent and 9.2 gram for chromium content between 0.1 and 0.2 per cent. Dissolve the sample in 10 cc. of sulfuric acid and when solution is complete add 0.5 cc. of nitric acid (sp. gr. 1.42) and heat until dense fumes are evolved. Cool, dilute with a little water, add 50 cc. of 2.5 N sodium hydroxide solution and 1 gram of sodium peroxide, and boil 5 minutes or until the excess of peroxide is destroyed. Cool to room temperature, transfer to a 200 cc. volumetric flask, dilute to the mark, mix, and filter off 100 cc. To this aliquot part add 5 cc. of the diphenylsemicarbazide reagent, 10 cc. of sulphuric acid, mix, and compare the color with that of a standard.

**Note.**—Small amounts of 0.01 N  $K_2Cr_2O_7$  solution added to solutions containing 4 grams of dissolved electrolytic iron gave colors which compared with that obtained with similar solutions containing no iron. The chromium content varied from 0.004 to 0.0017 per cent and the results obtained were accurate to about 0.0001 per cent.<sup>2</sup>

#### DETERMINATION OF CHROMIUM AS CHROMATE

(Oxidation by  $(NH_4)_2S_2O_8$  and  $AgNO_3$ )

This method depends upon the oxidation of a small amount of chromium to chromate by persulfate and silver nitrate and comparing the yellow color thus produced with a standard chromate solution. It is especially recommended for the estimation of chromium in rocks and ores but may also be adapted to the analysis of steels.

##### Reagents.

1. Sulfuric acid, 6 N.
2. Nitric acid, 6 N.
3. Ammonium hydroxide, 6 N.
4. Sodium carbonate, 6 N.

<sup>2</sup> B. S. Evans, *Analyst*, **46**, 285 (1921).

5. Sodium thiosulfate. Use a concentrated solution.
6. Silver nitrate, 1 N.
7. Sodium or ammonium persulfate.
8. Sodium phosphate,  $\text{Na}_2\text{HPO}_4$ , 1 N.
9. Ethyl alcohol or methyl alcohol.
10. Standard chromate solution. Dissolve 0.2555 gram of potassium chromate,  $\text{K}_2\text{CrO}_4$ , in water, dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of  $\text{Cr}_2\text{O}_3$ . If a more dilute standard is required, dilute 50 cc. of the latter to 250 cc. and mix. This solution contains 0.02 mg. of  $\text{Cr}_2\text{O}_3$  per cubic centimeter.

**Procedure.**—Dissolve the sample (Cr content not less than 2 mg.) in dilute sulfuric acid and nearly neutralize the excess acid with sodium carbonate solution. Precipitate the chromium and manganese by the addition of a concentrated solution of sodium thiosulfate. Filter and dissolve the precipitate in nitric acid. Add 3 cc. of silver nitrate solution and a few crystals of sodium or ammonium persulfate. Warm the solution until the maximum depth of color is obtained. If manganese is present, it will appear at this stage as permanganate and will mask the yellow color of the chromate. The permanganate may be destroyed by heating the solution with methyl or ethyl alcohol,<sup>3</sup> or by heating with ammonia.<sup>4</sup> In case iron is present, it is removed by adding sodium phosphate. The precipitate is filtered off and the filtrate matched in color against that of a standard chromate solution. Any of the usual methods may be employed for the comparison.

#### Notes.

1. A sample containing at least 2 mg. of chromium must be used, since experiment has shown that the error is too great in the oxidation of a smaller amount. The solution may later be diluted and an aliquot part used for the color comparison.
2. Other metals may be precipitated by the sodium carbonate along with the chromium and manganese but they do not interfere with the analysis.
3. The silver nitrate acts as a catalyzer in the oxidation of the chromium and manganese by persulfate.

<sup>3</sup> W. F. Hillebrand, U. S. Geol. Surv., Bull. 700, p. 182 (1919).

<sup>4</sup> M. Dittrich, Z. anorg. Chem., **80**, 171 (1913).

4. The permanganate and silver are precipitated as hydroxides by ammonia but the chromate is unchanged.

5. If manganese is absent, the treatment with alcohol or ammonia is omitted.

#### DETERMINATION OF CHROMIUM AS DICHROMATE

(Oxidation by  $\text{KMnO}_4$ )

This method<sup>5</sup> is based upon the oxidation of chromium to chromate by permanganate and comparison of the resulting yellow chromate solution with that of a standard solution. It is especially adapted to the estimation of chromium in steels.

##### Reagents.

1. Acetic acid, 6 N.
2. Sulfuric acid, 6 N and 9N.
3. Nitric acid, sp. gr. 1.42.
4. Sodium hydroxide, 5 N.
5. Diammonium hydrogen phosphate,  $(\text{NH}_4)_2\text{HPO}_4$ .
6. Potassium permanganate. Use a saturated solution.
7. Manganese sulfate,  $\text{MnSO}_4$ , 5 per cent.
8. Standard dichromate solution. Use a 0.01 N potassium dichromate,  $\text{K}_2\text{Cr}_2\text{O}_7$ , solution.

**Procedure.**—Dissolve about 4 grams of the metal in 50 cc. of 6N  $\text{H}_2\text{SO}_4$ , add 1 cc.  $\text{HNO}_3$  (sp. gr. 1.42) to oxidize the iron, and boil until all the  $\text{HNO}_3$  has been removed. Add 25 grams of  $(\text{NH}_4)_2\text{HPO}_4$  and dilute with 250 cc. of water. If a precipitate forms, add more  $\text{HNO}_3$ . Heat the solution to boiling and add drop by drop a saturated solution of  $\text{KMnO}_4$  until a permanent precipitate or red color is obtained, then add 12 drops more and boil 15 minutes. Meanwhile place 120 cc. of 5 N  $\text{NaOH}$  in a beaker, add to it 14 drops of saturated  $\text{KMnO}_4$  solution and boil on a hot-plate several minutes, adding drop by drop more  $\text{KMnO}_4$  if necessary to give a purple color. After the solution of the steel has boiled 15 minutes, the sodium hydroxide solution is removed from the hot-plate, 10 cc. of 5 per cent  $\text{MnSO}_4$  solution is added to destroy the permanganate, and the acid solution of the steel poured into the alkali with stirring. The solution is then

<sup>5</sup> B. S. Evans, Analyst, **46**, 38 (1921).



transferred to a 500 cc. volumetric flask. Be certain that it is alkaline. Now add 10 cc. of acetic acid and test to be sure that the solution is now acid. Finally, dilute to the mark, thoroughly mix, and filter through a dry filter. Place 100 cc. of the filtered solution in a Nessler cylinder. Into another cylinder place 80 cc. of water, 20 cc. of 9 N  $\text{H}_2\text{SO}_4$ , and add 0.01 N  $\text{K}_2\text{Cr}_2\text{O}_7$  drop by drop, with mixing, until the color matches that of the 100 cc. aliquot part of the sample.

**Notes.**

1. In case cobalt or nickel is present, pour 100 cc. of solution into a flask, heat to boiling, add NaOH to precipitate  $\text{Co}(\text{OH})_2$  and  $\text{Ni}(\text{OH})_2$ , cool, filter, allowing the filtrate to run into a Nessler cylinder, and match the color as described above.

2. When the chromium content is very small, an excess of about 0.25 cc. of 0.01 N  $\text{K}_2\text{Cr}_2\text{O}_7$  will be used. The exact amount should be determined by a "blank" test.

## CHAPTER XV

### COBALT

#### DETERMINATION OF COBALT BY EXTRACTION WITH AMYL ALCOHOL

THIS method is based upon the fact that amyl alcohol or ether takes up cobalt forming a blue solution and leaves nickel in the water layer.

##### Reagents.

1. Ammonium thiocyanate, 30 per cent solution.
2. Sodium pyrophosphate. Solid.
3. Amyl alcohol.
4. Standard cobalt solution. Dissolve 0.4926 gram of cobalt nitrate,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , in water, dilute to a liter and thoroughly mix. One cubic centimeter of the solution contains 0.1 mg. of cobalt.

**Procedure.**—The amount taken for analysis should not contain over 0.5 mg., or if desirable a larger sample may be taken and an aliquot part used. The sample is dissolved in 30 per cent ammonium thiocyanate, if possible, otherwise dissolve in the least possible amount of acid and then add ammonium thiocyanate until the concentration of the latter is at least 25 per cent, adding some of the solid salt if necessary. If the sample is a liquid, add solid ammonium thiocyanate sufficient to give a 30 per cent solution. Next add about 0.5 gram of sodium pyrophosphate, transfer the solution to a separatory funnel, add 25 cc. of amyl alcohol, extract as much as possible by thorough soaking, separate the blue layer of amyl alcohol cobalt solution and put it in a 50 cc. Nessler cylinder. Make a second extraction with 25 cc. of amyl alcohol and add the extract to the Nessler cylinder. Usually two extractions are sufficient to remove practically all of the cobalt. Compare the amyl alcohol extract against a standard prepared by extracting a known quantity of cobalt in amyl alcohol in the same way as was done in the case of the sample.

### Notes.

1. For complete extraction of the cobalt the minimum concentration of ammonium thiocyanate is 25 per cent.<sup>1</sup>

2. The addition of sodium pyrophosphate entirely prevents the iron from reacting with the ammonium thiocyanate and, hence, the cobalt can be extracted directly without adding sodium carbonate to precipitate the iron. The sodium pyrophosphate also insures a very uniform color, which is not obtained when sodium carbonate is used due to the presence of a fine suspension of hydrous ferric oxide sufficient to give a greenish tinge to the solution and, hence, making it difficult to match.<sup>2</sup>

3. If the manganese content is high, it may form so much precipitate with the pyrophosphate as to make extraction impossible.

4. If iron and manganese are present, the same amounts of them should be added to the standard cobalt solution (before extraction) as contained in the sample.

5. The amyl alcohol extract cannot be filtered through paper before matching, owing to absorption of the colored salt by the paper.

### REFERENCE

J. W. Mellor, *Trans. Ceram. Soc. England*, 8, 132; *cf. ibid.*, 8, 125.

### DETERMINATION OF COBALT AS THE CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID

This method is based upon the blue color obtained when cobalt chloride is dissolved in concentrated hydrochloric acid. The procedure and precautions are the same as those for the determination of nickel as the chloride in concentrated hydrochloric acid. If nickel is absent, the comparison may be made by the method of dilution, diluting with concentrated hydrochloric acid. Should nickel be present, standard nickel solution is added to the cobalt standard until the tint of the solution is the same as that of the sample, after which dilution is made in the usual way.

### Reagents.

1. Hydrochloric acid, sp. gr. 1.19. The acid must be free of copper, cobalt, nickel, and iron.

<sup>1</sup> A. D. Powell, *J. Soc. Chem. Ind.*, **36**, 273 (1917).

<sup>2</sup> Powell, *loc. cit.*; *cf. G. Romijn, Pharm. Weekblad.*, **48**, 996.

2. Nitric acid, sp. gr. 1.42.

3. Standard cobalt solution. Dissolve 4.9261 grams of Cu-, Ni-, and Fe-free cobalt nitrate,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , in concentrated hydrochloric acid, repeatedly evaporate with concentrated hydrochloric acid as directed in the preparation of the standard nickel solution (page 304), and finally make up to 500 cc. with concentrated hydrochloric acid. This solution contains 2 mg. of cobalt per cc.

**Procedure.**—Same as for nickel, page 304.

### Notes.

1. Materials containing between 0.1 and 10 per cent of cobalt may be handled with a maximum error of  $\pm 5$  per cent.

2. For additional notes, see those for nickel, pages 304 and 305.

### DETERMINATION OF COBALT BY $\alpha$ NITROSO $\beta$ -NAPHTHOL

$\alpha$ -Nitroso- $\beta$ -naphthol added to a solution containing cobalt gives a red color the intensity of which is proportional to the amount of the metal present. The following procedure<sup>3</sup> is especially adapted to the analysis of materials such as varnish and zinc oxide paints.

### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.

2. Hydrochloric acid, 6 N.

3. Ammonium hydroxide, 6 N.

4. Ammonium citrate reagent. Dissolve 500 grams of citric acid in 250 cc. of water and add 500 cc. of ammonium hydroxide (sp. gr. 0.88). This solution contains an excess of ammonia.

5.  $\alpha$ -Nitroso- $\beta$ -naphthol.<sup>4</sup> Boil 0.1 gram of  $\alpha$ -nitroso- $\beta$ -naphthol with 20 cc. of water and 1 cc. of dilute sodium hydroxide solution, filter, and dilute to 200 cc.

6. Standard cobalt solution. Dissolve 0.4926 gram of cobalt nitrate,  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , in water and dilute to a liter. Mix thoroughly. One cubic centimeter contains 0.1 mg. of cobalt. For the balancing method, 50 cc. of the standard solution are treated with double the quantities of reagents specified for the sample and then

<sup>3</sup> E. G. Jones, *Analyst*, **43**, 317 (1918); cf. F. W. Attack, *J. Soc. Chem. Ind.*, **34**, 641 (1915).

<sup>4</sup> F. W. Attack, *J. Soc. Chem. Ind.*, **34**, 641 (1915).

diluted to 500 cc. and mixed. This solution contains 0.01 mg. of cobalt per cubic centimeter. The solution should be used at once, since after a short time a precipitate appears.

**Procedure.**—Weigh out a sample of such size as to contain between 0.05 and 2 mg. of cobalt and incinerate in a porcelain crucible. Extract the ash with concentrated hydrochloric acid. If any insoluble matter remains, add aqua regia and finally drive off the nitric acid by adding concentrated hydrochloric acid and heating. Evaporate the solution to dryness, take up the residue in hot water and a few drops of 6 N hydrochloric acid and dilute to a convenient volume in a volumetric flask. Place an aliquot part of the solution in a Nessler cylinder, add 5 cc. of ammonium citrate reagent, dilute to about 90 cc., add 5 cc. of  $\alpha$ -nitroso- $\beta$ -naphthol, dilute to 100 cc. with water, mix, and match against a standard solution of cobalt prepared by adding the same amounts of ammonium citrate and  $\alpha$ -nitroso- $\beta$ -naphthol as used with the sample. Comparisons are made by the balancing or dilution methods, or the method of duplication.

#### Notes.

1. The above procedure must be modified if copper or a minute trace of nickel or of manganese is present.

The copper is removed by passing hydrogen sulfide into the slightly acidified solution (about 0.05 N with HCl). Filter, boil off the hydrogen sulfide from the filtrate, and complete the analysis in the usual way.

If nickel is present, add a few cubic centimeters of ammonium citrate reagent to the neutral or slightly acid solution, dilute to about 100 cc. with water and heat almost to boiling. Then add a 1 per cent solution of dimethylglyoxime in alcohol (1 cc. for every 2 mg. of Ni) and make slightly alkaline by adding ammonium hydroxide drop by drop. Stir the mixture, allow it to stand about 5 minutes in a warm place, filter into a volumetric flask and wash with hot water. Cool the solution, dilute to the mark, and mix. Evaporate an aliquot part to dryness in a porcelain dish and gently ignite the residue. Cool, treat first with aqua regia, then with concentrated hydrochloric acid, and evaporate to dryness. Moisten with a drop of 6 N hydrochloric acid, extract with water and complete the cobalt determination in the usual way.

When the amount of manganese is considerably greater than that of cobalt, it must be removed, for the most part at least. The solution

of the sample containing the metals as chlorides is digested on a hot-plate with an equal volume of nitric acid (sp. gr. 1.2) and a little sodium bismuthate. As soon as the pink color of the permanganate has disappeared, filter, dilute the filtrate to a suitable volume, and transfer an aliquot part to a Nessler cylinder. Add 5 cc. of *neutral* ammonium citrate, neutralize the solution with ammonium hydroxide (litmus paper) and then add a measured quantity of 6 N ammonium hydroxide. Compare the color with a standard cobalt solution prepared by adding the same amounts of neutral ammonium citrate, ammonium hydroxide, and  $\alpha$ -nitroso- $\beta$ -naphthol. The comparison may be made by the methods of dilution, balancing, or duplication.

2. The most satisfactory amount of cobalt for comparison is about 0.1 mg.<sup>5</sup>

3. When nickel is removed by precipitation as dimethylglyoxime, the excess of the latter is removed by evaporating to dryness and treating the residue with aqua regia.

4. The purpose of adding ammonium citrate is to prevent the interference of metals other than those of copper, nickel, and manganese.

5. A pure solution of  $\alpha$ -nitroso- $\beta$ -naphthol does not keep satisfactorily but its sodium salt does.

6. A series of standard solutions is not satisfactory as the cobalt precipitates after a short time.

7. The accuracy of the method for the estimation of cobalt in varnishes and zinc oxide paints is shown by the following results obtained by Jones<sup>6</sup> who added known amounts of cobalt to the materials.

#### VARNISHES

Per cent Co added.....	0.0095	0.039	0.066	0.072	0.104	0.180
Per cent Co found.....	0.009	0.037	0.062	0.070	0.103	0.178

#### ZnO—PAINTS

Per cent Co added.....	0.063	0.114				
Per cent Co found.....	0.063	0.103				

<sup>5</sup> E. G. Jones, *loc. cit.*

<sup>6</sup> *Loc. cit.*

## CHAPTER XVI

### COPPER

#### DETERMINATION OF COPPER BY AMMONIA

This determination is based upon the blue copper ammonia complex ion,  $[\text{Cu}(\text{NH}_3)_4]^{++}$ , which forms when an excess of ammonia is added to a solution of cupric ions.

##### Reagents.

1. Ammonium hydroxide, sp. gr. 0.90, and dilute ammonium hydroxide, 3 N.

2. Nitric acid, sp. gr. 1.42.

3. Sulfuric acid, sp. gr. 1.84.

4. Permanent standards. Dissolve about 0.3 gram of pure copper in 5 cc. of conc. nitric acid and 5 cc. of conc. sulfuric acid and evaporate until dense white fumes of the latter are evolved. Then cool, add 25 cc. of distilled water and conc. ammonia until a clear blue solution is obtained, finally diluting with 3 N ammonium hydroxide until 1 cc. of solution contains exactly 0.0025 gram of copper. Accurately measure out portions of the standard containing from 0.10 to 1.30 per cent of copper, place them in clear glass cylinders of uniform size and fitted with ground-glass stoppers, dilute to the 200 cc. mark with 3 N ammonium hydroxide, and thoroughly mix. If these standards are kept in a cool place and away from direct sunlight they will last a long time. (See Note 2.)

**Procedure.**—Two and a half grams of the substance are placed in a casserole,<sup>1</sup> 15 cc. of conc. nitric acid and 5 cc. of conc. sulfuric acid are added, and the mixture heated to a thick pasty mass. Dissolve the copper sulfate in 70 cc. of water, add 30 cc. of conc. ammonia, filter, and wash the residue twice with dilute ammonia (1 : 10). Rinse the residue back into the casserole with 50 cc. of water, being careful not

<sup>1</sup> G. L. Heath, J. Am. Chem. Soc., **19**, 24 (1897).

to damage the filter, and add sulfuric acid sufficient to dissolve the ferric oxide and alumina. Twenty-five cubic centimeters of conc. ammonia are then added and the solution filtered and washed as before. The filtrate and washings are mixed with the main filtrate, the solution transferred to a glass cylinder like those used for the standards, diluted to the 200 cc. mark with 3 N ammonium hydroxide, thoroughly mixed, and compared with the series of standards. The matching is best made by placing a piece of plain white paper behind the sample and standard cylinders and holding them against a window pane.

### Notes.

1. Copper is usually to be estimated in tailings or lean blast furnace slags and must be separated from silica, and the oxides of iron, aluminum, and calcium. The above method of double precipitation of the iron and aluminum is more accurate, and requires less time than either the precipitation by aluminum or a single precipitation by ammonia.

2. Nitric acid used to dissolve the pure copper for the standard is replaced by sulfuric acid and the solution made strongly ammoniacal with ammonia. This procedure gives standard ammoniacal copper solutions which are permanent for a long time, provided they are kept tightly stoppered and away from direct sunlight.

3. Should the first residue be evaporated too dry, or should the color be very purple after filtration, the separation of copper from basic oxides by a single precipitation with ammonium hydroxide will be incomplete. Should the solution have a greenish tint, the readings are likely to be a little too high, but the error is within the limits of error in sampling. In case the green tint is very pronounced the analysis should be repeated. If cupri-ferrous material is present the weighed sample should be heated several minutes in a porcelain crucible at a bright red heat. Stir with a platinum wire during the heating.

4. Results with samples containing from 25 to 35 per cent<sup>2</sup> of iron and aluminum oxides showed from 0.00 to 0.04 per cent of copper in the residue on the filter after the second precipitation.

5. Although both copper sulfate and copper nitrate give a dark blue solution in ammonia, the colors are not the same for the same copper

<sup>2</sup>Heath, *loc. cit.*



content. Hence, the standard and the sample must consist of the same copper salt.

6. The analysis is most sensitive when the copper content is 0.0127 mg. per cubic centimeter. At this concentration, the addition of 0.0716 mg. of copper can be detected.<sup>3</sup>

7. If the method of balancing or that of dilution is to be used, prepare a standard ammoniacal copper solution containing 0.01 mg. of copper per cubic centimeter.

#### DETERMINATION OF COPPER AS THE SULFIDE

The addition of a little hydrogen sulfide water to a solution containing a minute quantity of copper gives a colloidal dispersion of copper sulfide whose brown color may be quantitatively matched against a standard prepared in a similar way.

##### Reagents.

1. Nitric acid, sp. gr. 1.42.
2. Hydrogen sulfide water. Saturate distilled water with pure hydrogen sulfide prepared as directed on page 240.
3. Ammonium chloride. Use a saturated solution.
4. Sodium acetate, 1 N.
5. Sugar solution. Use a 50 per cent solution of pure white sugar.
6. Standard copper solution. Dissolve 0.3928 gram of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in water, dilute to a liter, and mix. This solution contains 0.1 mg. of copper per cubic centimeter.

**Procedure.**—Dissolve a weight of sample that contains not more than 0.00025 gram (better 0.0001 gram) of copper, or if a larger amount is taken, then use an aliquot part of the solution. Add to the dissolved sample, or aliquot part, 3 cc. of sodium acetate solution, 5 cc. of ammonium chloride solution, and 3 cc. of conc. nitric acid. Then add 2 cc. of hydrogen sulfide water and thoroughly mix. Match the brown colored suspension against a standard by any of the methods except that of a series of standards. If the dilution or balancing method is used, 10 cc. of the standard copper solution are treated with the same reagents as the sample, except that 6 cc. of hydrogen sulfide water are used instead of 2 cc., and the final solution diluted to 100 cc. One cubic centimeter of this solution contains 0.01 mg. of copper.

<sup>3</sup> F. D. Snell, *Colorimetric Analysis*, p. 45. D. Van Nostrand Co., New York, 1921.

To compare by duplication, dilute the sample to 50 cc. and add the standard copper solution, drop by drop, to 25 cc. of water containing the reagents used in the sample, the final volume being brought to 50 cc. by the addition of water. Similar tubes must, of course, be used for the sample and standard.

#### Notes.

1. The presence of the ammonium chloride increases the delicacy very much.

2. Since the copper sulfide is in colloidal suspension, it may show a tendency to agglomerate and settle. This tendency may be largely overcome by taking a new sample and adding 5 cc. of a strong sugar solution, in addition to the other reagents, before adding the hydrogen sulfide water. The sugar acts as a peptizing agent.

3. Due to the copper sulfide being in the colloidal state, it is essential that strict attention be given to having the same quantities of electrolytes present in both the sample and the standard. The intensity of the color will vary with the size of the particles of copper sulfide and this in turn is influenced by electrolytes. Moreover, the order and manner of adding all reagents should be as nearly the same as practical when precipitating the copper in the sample and in the standard.

4. The test cannot be made in the presence of lead, silver, mercury, or bismuth since the sulfides of these metals color the solution brown. Iron, cobalt, and nickel are not likely to interfere since their sulfides do not form in acid solution. They would interfere, however, if present in very large amounts, due to the colored solutions given by their salts.

#### DETERMINATION OF COPPER AS THE CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID

Cupric chloride dissolves in strong hydrochloric acid to give a yellow solution. A maximum intensity in color is reached at a hydrochloric acid concentration of about 28 per cent.<sup>4</sup> Comparisons are, therefore, made in 28 per cent hydrochloric acid solution.

#### Reagents.

1. Hydrochloric acid. A 28 per cent solution is prepared as directed in the determination of iron by the chloride method, page 238.

<sup>4</sup> C. Hüttner, *Z. anorg. Chem.*, **86**, 341 (1914).

2. Nitric acid. Use the C.P. acid, sp. gr. 1.42, if it gives a zero "blank," otherwise, redistill the concentrated acid.

3. Standard copper solution. Dissolve 1.0000 gram of pure copper in nitric acid, add strong hydrochloric acid, and evaporate to dryness. Repeat this treatment twice, dissolve the final residue in a little hydrochloric acid, and then dilute to one liter with the 28 per cent acid. One cubic centimeter of this solution contains 0.001 gram of copper.

**Procedure.**—Dissolve the weighed sample, containing about 0.01 gram of copper (or about 0.1 gram), in concentrated hydrochloric acid of about 28 per cent strength. Dilute to 100 cc. with the 28 per cent acid (or to 1000 cc. if 0.1 gram sample was used). If the substance is not readily dissolved in hydrochloric acid, dissolve it in conc. nitric acid or aqua regia. Replace the nitric acid by repeated evaporation to dryness (three times) with hydrochloric acid, finally dissolving the residue in hydrochloric acid and diluting the solution to 100 cc. (or to 1000 cc. if 0.1 gram sample was used) with the 28 per cent acid. Compare 50 cc. of the diluted sample with the standard by the method of balancing.

#### Notes.

1. Manganese and free chlorine do not impair the accuracy of the determination. Cobalt and nickel do not interfere unless they are present in large amounts. Nitric acid and the oxides of nitrogen color the solution yellow and, hence, must be removed by repeated evaporation to dryness with strong hydrochloric acid. Iron also gives a yellow color in hydrochloric acid solution. The ratio of intensities of the color of the copper solution to that of the iron solution is 5 : 9. The copper may be separated from the iron<sup>5</sup> (also from cobalt and nickel) by precipitating as the sulfide. The cupric sulfide is then dissolved by boiling in dilute nitric acid, the sulfur filtered off, the filter thoroughly washed, and the filtrate together with the washings evaporated to dryness with strong hydrochloric acid. Continue the analysis as directed in the procedure above.

2. The hydrogen sulfide used to precipitate the copper must be thoroughly washed. It is conveniently prepared as directed on p. 240.

3. Small quantities of organic matter may give a yellow color to strong hydrochloric acid. Hence, any organic matter present must

<sup>5</sup> C. Hüttner, *loc. cit.*

be removed by igniting the sample in the presence of air. The ignited mass is then brought into solution in the usual way.

4. The estimation of copper as chloride in concentrated hydrochloric acid is as accurate as the ammonia method.

5. The method of balancing is preferable if a large number of analyses are to be made, owing to the cost of the large amount of hydrochloric acid required for the method of dilution. All dilutions must be made with 28 per cent acid.

#### DETERMINATION OF COPPER AS THE BROMIDE

This method is based upon the brown color of cupric bromide in acid solution.<sup>6</sup>

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Potassium bromide. Dissolve 100 grams of pure potassium bromide in 150 cc. of boiled distilled water, dilute to 200 cc., and mix. Keep in a brown glass bottle and in the dark.
3. Standard copper solution. Place 0.3928 gram of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in a liter volumetric flask, dilute to the mark with water, and mix. One cubic centimeter of the solution contains 0.1 mg. of copper.

**Procedure.**—Dissolve the weighed sample in water or acid, dilute to a definite volume, mix thoroughly, and measure out accurately 5 cc. Twenty cubic centimeters of the potassium bromide solution are placed in a beaker, 10 cc. of conc. sulfuric acid are added a little at a time, and the precipitated potassium sulfate filtered off. Five cubic centimeters of the filtrate are added to the 5 cc. aliquot part of the sample, together with 2 cc. of conc. sulfuric acid. After thorough mixing, the solution is matched in color against a series of standard copper solutions prepared in a similar way.

##### Notes.

1. The bromide method may be used to estimate small amounts of copper in chlorides, nitrates, and sulfates. It may also be used when the copper is present in a complex radical, provided the complex is decomposed by treating with a concentrated acid.

<sup>6</sup> Bull. soc. pharm. Bordeaux, Aug.-Dec., 1915.

2. The potassium bromide solution is unstable and should be prepared fresh at least every two weeks. It must be kept in the dark and in a brown bottle. Prepare only a small volume of the reagent as needed, if determinations are made infrequently.

3. The standard color solutions are fairly stable and may be kept for some time.

#### DETERMINATION OF COPPER BY POTASSIUM FERROCYANIDE

When potassium ferrocyanide is added to solutions containing copper ions a purplish brown color is produced, due to the formation of the corresponding copper complex salt,  $\text{Cu}_2\text{Fe}(\text{CN})_6$ . The intensity of the color serves as a measure of the copper content of the solution.

##### Reagents.

1. Nitric acid, sp. gr. 1.42.
2. Sulfuric acid, sp. gr. 1.84.
3. Ammonium hydroxide, 6 N.
4. Ammonium chloride, saturated solution.
5. Potassium ferrocyanide, 3 per cent solution.
6. Standard copper solution. Dissolve 0.3928 gram of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , dilute to a liter and thoroughly mix. One cubic centimeter of this solution contains 0.1 mg. of copper.

**Procedure.**—The weighed sample, containing between 0.00005 and 0.005 gram of copper, is dissolved in water or nitric acid. If the latter, boil till most of the acid is driven off, add 5 cc. of sulfuric acid and re-boil. Cool, dilute a little, filter, and wash the residue with warm water. Add ammonia to the combined filtrate and washings until slightly alkaline and boil off the excess of ammonia, then add 5 drops of the potassium ferrocyanide solution and compare the color against a standard by the method of dilution or that of duplication. For the method of duplication the sample is diluted to 50 or 100 cc., thoroughly mixed and one-half of it used for the comparison. The standard is made by adding 5 drops of potassium ferrocyanide solution to 35 cc. of water containing 5 cc. of ammonium chloride, mixing, and adding the standard copper solution until the color matches that of the sample when carefully diluted to the same volume as that of the sample. Both standard and sample are made up in similar tubes.

If the dilution method is to be used, take 10 cc. of the standard, add 25 cc. of water, 5 cc. of ammonium chloride, 5 drops of potassium ferro-

cyanide solution, and dilute to 50 cc. Then dilute slowly until the color matches that of the sample, taking the precaution to mix the solution thoroughly after each dilution.

### Notes.

1. The presence of ammonium chloride, ammonium nitrate, or potassium nitrate increases the delicacy of the test. With these salts present, 1 part of copper can be detected in 2,500,000 parts of solution.

2. Potassium ferrocyanide gives a white precipitate with lead, but the amount of lead likely to be present at the dilution of the test is usually negligible. For more accurate work, the same amount of lead present in the sample may be added to the standard in the form of lead nitrate.<sup>7</sup>

3. A large amount of iron interferes with the analysis. The iron is removed by precipitating with ammonia and filtering off the ferric hydroxide. The latter may be used to estimate the iron content of the sample. In case there is a large precipitate, dissolve it in a little hydrochloric acid, reprecipitate with ammonia, filter, and add the filtrate to the first one. The resolution and reprecipitation is to recover the small amount of copper carried down with the first precipitate.

4. If a large amount of zinc is present it is removed as follows: Slightly acidify with acetic acid the filtrate from the iron precipitation, add 5 cc. of 8 per cent disodium ammonium phosphate,  $\text{Na}_2\text{NH}_4\text{PO}_4$ , solution; boil, cool, filter, and treat the filtrate for copper in the usual way.

5. The test solution must be *neutral*. In acid solution an earthy brown color is formed; in an alkaline solution the color fades. Litmus paper should be used as an indicator.

6. The color fades too quickly for the use of a series of standards and is not suitable for comparison by balancing.

### DETERMINATION OF COPPER BY SALICYLIC ACID

#### Reagents.

1. Salicylic acid. One-half per cent solution in dilute alcohol.
2. Acetic acid. Ten per cent solution of iron-free acetic acid.
3. Potassium nitrate. Two per cent solution of the pure salt.

<sup>7</sup> J. Ind. Eng. Chem., **7**, 1035 (1915).

4. **Standard copper solution.** Dissolve 0.3928 gram of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in water, dilute to a liter and mix thoroughly. One cubic centimeter of the solution contains 0.1 mg. of copper.

**Procedure.**—The sample is dissolved in water and diluted to 10 cc. in an ordinary test tube. To each of a series of test tubes (selected to match, as nearly as practicable, the sample tube) is added measured amounts of the standard copper solution. The amounts of the standard should vary from 0.1 to 1.0 cc. and each is diluted to 10 cc. Then to each tube, including the sample, is added 5 drops of potassium nitrate solution, 5 drops of acetic acid solution, and, finally, 3 cc. of the salicylic acid. Mix the contents of each tube, heat them to boiling in a water-bath for 45 minutes, and compare the sample with the standards. The reading is taken as that standard which matches the closest the color of the sample tube.

#### Notes.

1. This method is sensitive to 0.01 mg. of copper.
2. The method cannot be used in the presence of free mineral acids, citric and tartaric acids, or iron salts.<sup>8</sup>
3. The color produced by the reaction of salicylic acid with copper ions fades fairly rapidly. Hence, comparison is always made with a series of standards prepared simultaneously with the sample.

#### DETERMINATION OF COPPER BY ETHYL XANTHATE

This determination is based upon the yellow color produced when potassium ethyl xanthate is added to a solution containing a minute quantity of copper.<sup>9</sup>

#### Reagents.

1. Potassium ethyl xanthate. Use 1 gram of the pure salt per liter. Keep the solution in an amber-colored glass-stoppered bottle.
2. Standard copper solution. Dissolve 0.3928 gram of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , dilute to a liter and mix. One cubic centimeter of the standard contains 0.1 mg. of copper.

<sup>8</sup> Z. Nahr. Genussm., **22**, 727.

<sup>9</sup> W. W. Scott, Standard Methods of Chemical Analysis, 4th ed., p. 197. D. Van Nostrand Co., New York, 1925.

**Procedure.**—Weigh out a sample to contain not more than 0.001 gram of copper, dissolve in water, dilute to 90 cc. and add 10 cc. of potassium ethyl xanthate. Compare the yellow color produced with that of a standard. For the method of duplication, the standard is made by adding 10 cc. of the potassium ethyl xanthate solution to 60 cc. of water and running in the standard copper solution, little by little, till the color matches the sample after their volumes have been made equal.

To match the color by balancing or diluting, 10 cc. of the standard copper solution are diluted to 500 cc. with water and 10 cc. of the potassium ethyl xanthate solution. This solution has a copper content of 0.002 mg. per cubic centimeter.

**Notes.**

1. The ethyl xanthate method is excellent for the estimation of copper present as impurity in salts.
2. Small amounts of lead, iron, cobalt, nickel, manganese, and zinc do not interfere with the accuracy of the test.
3. Not more than 0.001 gram of copper must be present in the sample taken for analysis. If more than this amount is present, copper xanthate precipitates.
4. If the sample is insoluble in water, dissolve in nitric acid and displace the nitric acid by evaporating the solution to dryness with hydrochloric acid. The residue is taken up in water and the analysis continued in the usual way. Any organic matter in the sample must be removed. This may be accomplished by igniting the sample with a little sodium or potassium nitrate and removing the excess of nitrate by adding hydrochloric acid and evaporating to dryness.



## CHAPTER XVII

### FLUORINE

#### DETERMINATION OF FLUORINE BY ITS BLEACHING ACTION ON AN OXIDIZED TITANIUM SOLUTION <sup>1</sup>

FLUORINE has a powerful bleaching action on the yellow color produced by oxidizing a titanium solution with hydrogen peroxide. By comparing the colors of equal volumes of two solutions containing the same amount of titanium oxidized by hydrogen peroxide, one containing fluorine and the other none, the percentage of fluorine can be *calculated*.

##### **Reagents.**

1. Sulfuric acid, 6 *N*.
2. Hydrogen peroxide, 3 per cent. Titrate the peroxide against a standard potassium permanganate solution from time to time to be certain its strength is still satisfactory.
3. Fusion mixture of sodium and potassium carbonates.
4. Ammonium carbonate.
5. Standard titanium sulfate solution. Dissolve potassium titanium fluoride in water, add a large excess of sulfuric acid; evaporate until sulfur trioxide is given off, cool, add more water, and repeat the operation several times to insure complete removal of fluorine. Dilute with a large volume of water and determine the titanium content of the solution gravimetrically by precipitating a measured portion of it with ammonium hydroxide, filtering, igniting the precipitate, and weighing the  $\text{TiO}_2$ . Dilute the remainder of the solution so that 1 cc. contains 0.1 mg. of  $\text{TiO}_2$ , at the same time adding enough sulfuric acid to make a 3 per cent solution.
6. Standard fluorine solution. Dissolve 1.2419 grams of potassium zirconium fluoride (potassium fluorizirconite),  $\text{K}_2\text{ZrF}_6$ , in water, dilute to a liter and thoroughly mix. This solution contains 0.5 mg. of fluorine per cubic centimeter.

<sup>1</sup> G. Steiger, J. Am. Chem. Soc., **30**, 219 (1908); cf. H. E. Merwin, Am. J. Sci., **28**, 119 (1909).

**Procedure.**—For rocks containing only a few tenths of a per cent of fluorine, a 2-gram sample is satisfactory. The finely ground sample is fused with four or five times its weight of a mixture of sodium and potassium carbonates and the fused mass thoroughly bleached with hot water. Two grams of ammonium carbonate are added and the mixture heated on the water-bath for about 20 minutes. Allow the mixture to cool an hour or more and filter off the precipitate of iron, and aluminum hydroxides and silica. Evaporate the filtrate to a small volume (25 or 30 cc.) and again filter to insure a clear solution, allowing the latter to run into a 100 cc. volumetric flask. Add sulfuric acid to the solution until it is almost neutral, taking care not to add an excess, shake well until free from excess carbon dioxide, and then add sulfuric acid until the solution is slightly acid. Now add more acid dependent on the amount of fluorine present. For 0.01 gram of fluorine add 12 cc. of acid and for 0.00005 gram, 1 cc. of acid. For intermediate amounts of fluorine, use proportionate quantities of acid. To the acidified solution (or an aliquot part containing not more than 2 or 3 mg.) add 20 cc. of the standard titanium sulfate solution and 3 cc. of hydrogen peroxide, dilute to the mark with water and mix thoroughly. Compare this solution with a standard prepared by adding 3 cc. of hydrogen peroxide to 20 cc. of the standard titanium sulfate solution, diluting to 100 cc. with water and mixing. The comparison is made in a colorimeter whose error is not more than 2 or 3 per cent.

Since the two solutions contain the same amount of titanium per cubic centimeter their colors should be identical, but on account of the bleaching effect of fluorine on titanium solutions oxidized by hydrogen peroxide, the sample solution will be lighter in shade than the standard. The extent of bleaching is not directly proportional to the fluorine content, but by reference to the curve in Fig. 42, the amount of fluorine corresponding to a given bleaching can be found. Suppose, for example, a reading of 73.5 is obtained. The fluorine has caused a bleaching of the solution equivalent to 26.5 per cent of the titanium present. By finding 73.5 on the abscissa, with the help of the curve the amount of fluorine (0.0015 gram) can be read off directly on the ordinate.

#### Notes.

1. The above method will detect several hundredths of one per cent of fluorine and will approximate the quantity. Better results

are obtained with quantities up to several tenths of a per cent, and when not more than 2 per cent is present the results compare favorably with those obtained with the standard methods.

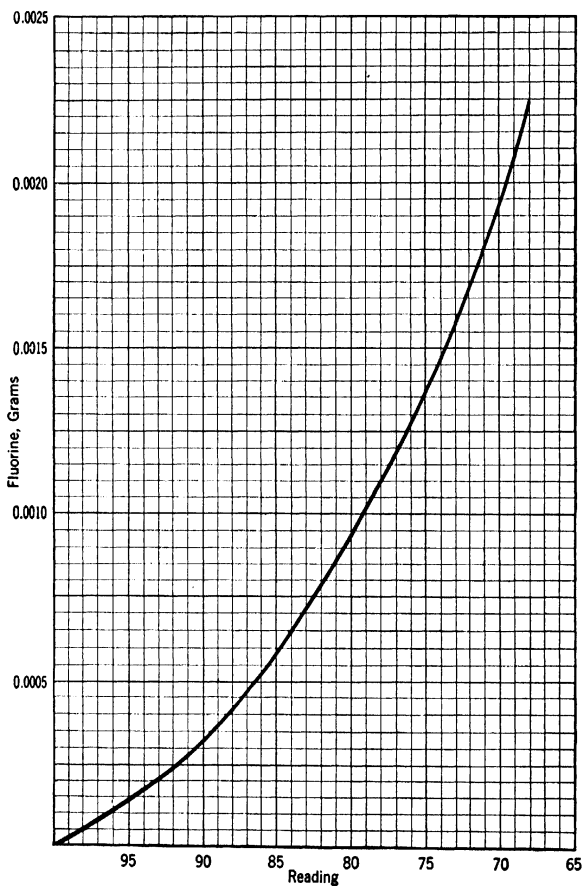


FIG. 42.

2. The ammonium carbonate treatment removes all the iron and aluminum and leaves not more than 30 mg. of silica. Iron prevents the bleaching action of the fluorine (and also colors the solution), but only a small amount of it is extracted from the fused mass, and

this is probably completely removed by the ammonium carbonate treatment. Aluminum, even in small amounts, has a very pronounced bleaching effect, but is completely removed by the ammonium carbonate treatment. Silica has only a slight effect when not over 0.1 gram is present. The 30 mg. or less of silica remaining after the ammonium carbonate treatment does not interfere. Phosphoric acid has a bleaching action on oxidized titanium solutions, but the amount of phosphorus likely to be present in a rock will not introduce a significant error. Sodium and potassium salts in large amounts have a slight bleaching action, but the quantity present in the above procedure does not seriously alter the results. The possible effect of traces of vanadates, tungstates, and chlorides has not been determined.

3. The color is affected by a change in temperature, an increase in intensity from 5 to 15 per cent being produced by a temperature rise of 30° C.

4. In shaking the solution until free from excess of carbon dioxide, "care should be taken not to have an excess of acid present before shaking, for the reason that the escaping gas will carry off some fluorine; even under the above conditions a slight loss occurs."<sup>2</sup>

5. Various mixtures, roughly representing commonly occurring rocks and containing accurately known amounts of fluorine, have been analyzed for fluorine by Steiger (*loc. cit.*) and serve to show the limits of accuracy of the above method. All of his results are given in Table XIV.

TABLE XIV

SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	MgCO <sub>3</sub>	CaCO <sub>3</sub>	NaH <sub>2</sub> PO <sub>4</sub>	TiO <sub>2</sub>	F. Calculated G	F. Found G
0.60	0.20	0.05	0.03	0.05	.....	.....	0.0114	0.0102
0.60	0.20	0.05	0.03	0.05	0.01	0.008	0.0052	0.00525
0.60	0.20	0.05	0.03	0.10	0.01	0.01	0.00284	0.0027
0.70*	0.15	0.05	0.05	0.05	0.01	0.005	0.0005*	0.0003
0.70	0.15	0.05	0.05	0.05	0.01	0.005	0.00526	0.0056
0.70	0.15	0.05	0.05	0.05	0.01	0.005	0.00526	0.0040
0.70	0.15	0.05	0.05	0.05	0.01	0.005	0.00253	0.0020
0.70	0.15	0.05	0.05	0.05	0.01	0.005	0.01228	0.0089
0.70	0.15	0.05	0.05	0.05	0.01	0.005	0.00516	0.00486
0.70	0.15	0.05	0.05	0.05	0.01	0.005	0.00536	0.00530

\* In this determination the fusion of the mixture was made as usual, and the fluorine added to the bleach water before the treatment with ammonium carbonate.

\* G. Steiger, *loc. cit.*

Steiger also made determinations of fluorine in natural rocks and compared them with the gravimetric results. His three sets of analyses are as follows:

	I Per Cent	II Per Cent	III Per Cent
Gravimetric determination.....	0.15	3.01	3.01
Colorimetric determination.....	0.21	2.58	3.20

Rocks containing between 10 and 20 per cent of fluorine were also analyzed, but the results were unsatisfactory, being in error by several per cent.

## CHAPTER XVIII

### GOLD

#### DETERMINATION OF GOLD BY DECOMPOSITION OF THE CYANIDE BY POTASSIUM BROMATE AND SULFURIC ACID <sup>1</sup>

A CYANIDE solution is treated with potassium bromate and concentrated sulfuric acid, and stannous chloride then added. As little gold as one cent per ton can be detected. Eight cents of gold per ton gives the purple of Cassius.

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Potassium bromate.
3. Stannous chloride. Ten per cent solution in dilute hydrochloric acid.

**Procedure.**—Add a small amount of potassium bromate to a 50 cc. solution of the sample and then concentrated sulfuric acid little by little until effervescence begins. The reaction will continue until all of the cyanide is decomposed. Boil off the bromine and add an excess of stannous chloride solution. Compare the color with a series of standards or from memory if the operator is sufficiently experienced with the test.

**Note.**—It is not necessary to boil off the bromine before adding stannous chloride, if the test is to be made at once. The color will not retain its true intensity for more than a minute unless the bromine has been removed.

#### DETERMINATION OF GOLD BY DECOMPOSITION OF THE CYANIDE BY SODIUM BROMIDE AND SODIUM PEROXIDE <sup>1</sup>

This method is similar to the preceding one except that sodium bromide is added to the sample in 50-cc. solution and then an excess of sodium peroxide. Bromine is liberated and the cyanide is decom-

<sup>1</sup> H. R. Cassel, Eng. Mining J., 76, 661 (1903).

posed. The solution is then neutralized with sulfuric acid, acidified with hydrochloric acid, stannous chloride solution added, and the color obtained compared with a series of standards or from memory.

#### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Hydrochloric acid, sp. gr. 1.19.
3. Sodium bromide.
4. Sodium peroxide.
5. Stannous chloride. Ten per cent solution in dilute hydrochloric acid.

#### DETERMINATION OF GOLD BY DECOMPOSITION OF THE CYANIDE BY AMMONIA<sup>2</sup>

This method differs from that of the two preceding ones only in the manner of reduction of the cyanide.

#### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Hydrochloric acid, sp. gr. 1.19.
3. Ammonium chloride. Ten per cent solution in dilute hydrochloric acid.

**Procedure.**—A solution of the sample is treated with concentrated ammonium hydroxide, an amount equivalent to one-third the volume of the solution being added. The solution is then neutralized with concentrated sulfuric acid, acidified with hydrochloric acid, stannous chloride added, and the color thus produced compared with standards similarly prepared.

#### DETERMINATION OF GOLD BY METAPHENYLENEDIAMINE

The yellow to brown color produced in this method is due to colloidal gold. As little as 0.05 per cent of gold can be estimated.

#### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Sulfuric acid, 6 N.
3. Metaphenylenediamine, 5 grams per liter.

<sup>2</sup>H. R. Cassel, *loc. cit.*

**Procedure.**—A solution of the gold in the form of cyanide is treated with concentrated hydrochloric acid. This decomposes the cyanide and converts the gold into its chloride.<sup>3</sup> Five cubic centimeters of the metaphenylenediamine are then added and the solutions mixed. Next add sulfuric acid, a little at a time, mixing after each addition, until the maximum intensity of color is produced. Compare with a series of standards similarly prepared.

**Note.**—The solution of metaphenylenediamine may turn pink on standing in the light. It may be decolorized by shaking with animal charcoal and filtering off the latter.

#### DORING'S METHOD FOR THE DETERMINATION OF GOLD<sup>4</sup>

This method is suitable for the estimation of gold in very poor ores. It will detect as little as a half gram of gold per ton of ore.

##### Reagents.

1. Bromine.
2. Ether.
3. Stannous chloride. Ten per cent solution in dilute hydrochloric acid.

**Procedure.**—Place 100 grams of the finely ground ore, slightly and uniformly moistened, in a ground glass-stoppered bottle, add 1 or 2 cc. of a 1 to 1 solution of bromine and ether, stopper the bottle and shake at frequent intervals for about 2 hours. Enough bromine must be present to keep the bottle filled with bromine vapor. Next add 50 cc. of water and let the mixture stand 2 hours with occasional shaking. Filter the solution, evaporate the filtrate to one-fifth of its original volume, add a little bromine water, and treat in a test tube with 10 cc. of stannous chloride solution.

##### Results.

0.1 per cent solution gives a deep brown color (opaque in thin layers).

0.01 per cent solution gives a brown violet at once (14 cm. column opaque).

0.001 per cent solution gives a pale violet at once. The color deepens on standing.

<sup>3</sup> J. A. Siemssen, Chem. Ztg., **36**, 934 (1912).

<sup>4</sup> Cf. F. D. Snell, Colorimetric Analysis, p. 91. D. Van Nostrand Co., New York, 1921.



0.0001 per cent solution, evaporated to one-fifth its former volume and a drop of bromine water added, gives a rose tint in a 14 cm. column.

0.00005 per cent solution, treated as the 0.0001 per cent solution, gives faint pink in a 14 cm. column.

The gold content of the ore may be calculated from the per cent of the solution.

#### DOWSETT'S METHOD FOR THE DETERMINATION OF GOLD<sup>4</sup>

The gold is extracted with sodium cyanide solution, precipitated with a few drops of lead nitrate solution and a small amount of zinc, the precipitate dissolved in nitric acid and stannous chloride solution added. Colors of solutions thus obtained vary from the palest yellow tinge to purple of Cassius, corresponding to gold contents from 2 cents per ton to 8 cents per ton, respectively.

##### Reagents.

1. Hydrochloric acid, 1.19.
2. Nitric acid, 1 acid to 2 water.
3. Sodium cyanide. Use a saturated solution.
4. Lead nitrate. Use a saturated solution.
5. Stannous chloride. Dissolve about 12.5 grams of stannous chloride crystals in 10 cc. of conc. hydrochloric acid and dilute with water to 100 cc.
6. Zinc dust. Use zinc dust sifted through a 200-mesh sieve.

**Procedure.**—The sample contained in about 500 cc. is poured into a liter bottle (which has very little shoulder), and 10 to 15 cc. of a saturated sodium cyanide solution and 2 or 3 drops of a saturated lead nitrate solution are added. Then add from 1 to 2 grams of zinc dust. Usually 1 gram is sufficient. Stopper the bottle and shake vigorously for 2 or 3 minutes, or until the precipitate is completely coagulated and will settle rapidly. Pour the contents of the bottle into a casserole, allow the precipitate to settle, and carefully decant the clear solution. Add hydrochloric acid to the residue, drop by drop, until the reaction stops, and then 1 or 2 drops more. Add 3 to 5 drops of dilute nitric acid and evaporate the solution to 1 or 2 cc. Transfer the solution to a small test tube, cool, add 1 cc. of stannous solution, and observe the color thus obtained.

<sup>4</sup> Met. Chem. Eng., 12, 460 (1914).

The colors and their corresponding gold values in cents per ton are as follows:

	Cents per ton
Very pale tinge.....	2
Pale yellow.....	3
Pale pinkish yellow.....	4
Deep pink.....	6
Purple of Cassius.....	8

### Notes.

1. If the gold content is very small, the tube should be allowed to stand 2 or 3 minutes to permit the full color to develop. A faint color is detected better by looking down the tube.

2. Different solutions may require some variation in the amounts of cyanide, lead nitrate, and zinc dust. The least amount of lead nitrate that will give a rapidly settling precipitate should be used. This will mean the minimum quantity of nitric acid will be required to dissolve the precipitate. Too much nitric acid must be avoided since it causes the color to differ from that specified.

3. If mercury is present, a dark coloration is obtained which interferes with the test.

### PRISTER'S METHOD FOR THE DETERMINATION OF GOLD \*

The gold is precipitated together with copper sulfide, the precipitate dissolved in alkaline cyanide, the solution treated with zinc dust, filtered, the excess zinc removed by hydrochloric acid, and the residue extracted with aqua regia. Upon diluting the extract with stannous chloride solution a colored solution is obtained. This is compared with similarly prepared standards unless the operator is familiar with the colors produced.

### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Nitric acid, sp. gr. 1.42.
3. Copper sulfate reagent. Boil for 10 minutes two parts of NaCl, 1 part of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , and 10 parts of water, in the presence of a small strip of copper. Cool and add a few drops of acetic acid.

\* J. Chem. Met. Min. Soc. S. Africa, 4, 235, 455.

4. Sodium sulfide, 3 N.
5. Potassium hydroxide, 6 N.
6. Potassium cyanide, 5 per cent.
7. Stannous chloride. 10 per cent solution in dilute hydrochloric acid.
8. Zinc dust. Use zinc dust that has passed a 200-mesh sieve.

**Procedure.**—Acidify a 200 cc. solution of the sample with hydrochloric acid and boil for several minutes. Add a slight excess of the copper sulfate reagent and a few drops of sodium sulfide solution. Boil the mixture about 5 minutes, let the precipitate settle, decant the clear liquid through a filter, and dissolve the precipitate on the filter and in the beaker in 3 cc. of potassium cyanide solution made alkaline with a few drops of potassium hydroxide. To the alkaline cyanide solution add 2 grams of zinc dust, heat to 45° and keep at this temperature about 30 minutes. Decant the mixture through a filter, dissolve the excess of zinc in hydrochloric acid, and then extract the residue with 10 cc. of aqua regia, passing the filtrate back through the filter several times to insure complete extraction. Add 10 cc. of stannous chloride solution to the extract, mix, and compare the color with a series of standards similarly prepared.

**Note.**—The solution of sample is first boiled several minutes with hydrochloric acid in order to decompose any cyanide present.

#### ROSE'S METHOD FOR THE DETERMINATION OF GOLD<sup>1</sup>

If a large volume of boiling water is poured into a stannous chloride solution, a yellowish white gelatinous precipitate of tin hydrates is formed. If the water contains a minute quantity of gold chloride the precipitate is colored purple (purple of Cassius). One part of gold per million parts of water will give at once a bright rose-colored precipitate when treated in this way. One part of gold in 100 millions gives a bluish purple color, markedly different from the color of the precipitate obtained with distilled water.

The method is good for the detection and estimation of gold in water and salts. Sea-water containing 1 part of gold in 20 millions shows a very distinct reaction. In this case the color is a black-rather than a purple-violet.

<sup>1</sup> T. K. Rose, Chem. News, 66, 271 (1892).

**Reagents.**

1. Stannous chloride. Use a saturated solution of stannous chloride in water acidulated with hydrochloric acid just sufficient to prevent hydrolysis.

2. Standard gold solution. Dissolve 0.00003 gram of gold (as chloride) in 3 liters of water. This solution contains 1 part of gold per 100 million.

**Procedure.**—The sample dissolved in 3 liters of water is heated to boiling and poured suddenly into a beaker containing 10 cc. of the stannous chloride solution, the two liquids being mixed as rapidly as possible. Compare the color of the precipitate with standards prepared in the same way. The operator should soon be able to estimate from memory the colors produced by standard amounts of gold.

## CHAPTER XIX

### HYDROGEN ION <sup>1</sup>

ACCORDING to the theory of electrolytic dissociation, all liquids, of which water is a constituent, contain free H and OH ions. When the number of H ions exactly equals the number of OH ions, the solution is said to be neutral. If the number of H ions exceeds that of the OH ions, the solution is said to be acid. Conversely, if the solution contains an excess of OH ions, it is said to be alkaline.

It is well known that acidity is due to the presence of H ions in a solution, the acidity increasing as the number of H ions increases. Strong acids are those which are highly dissociated in solution to give a large number of H ions. Weak acids are those which are but slightly dissociated in solution and, therefore, give relatively few H ions. For example, HCl is a strong acid and acetic acid is a weak acid. In titration 10 cc. of N/10 HCl and 10 cc. of N/10 acetic acid will, of course, each require 10 cc. of N/10 NaOH for neutralization. The number of H ions in N/10 HCl is, however, almost 70 times as great as the number of H ions in N/10 acetic acid. It is, therefore, readily seen that the H-ion concentration is not determined by simple titration. Since it is the H-ion concentration and not total acidity which is the controlling factor in the majority of chemical reactions, the importance of making H-ion determinations is apparent.

Pure distilled water will conduct an electric current to a very slight degree. This shows that a very small proportion of the water is dissociated into H and OH ions. By the *Mass Law*

$$\frac{\text{Concentration of H ions} \times \text{conc. of OH ions}}{\text{Concentration of undissociated H}_2\text{O}} = \text{a constant.}$$

Since the amount of undissociated water is relatively extremely

<sup>1</sup> The material in this chapter has been taken by permission from W. A. Taylor, *The A B C of Hydrogen Ion Control*, 4th ed., LaMotte Chemical Products Co., Baltimore, Md., 1928.

large, it can be taken as a constant, and the above equation, therefore, becomes

Conc. of H ions  $\times$  conc. of OH ions = a constant.

By electrical conductivity measurements, this constant has been found to be  $1/100,000,000,000,000$  or  $10^{-14}$  at  $22^\circ$  C. Since in pure distilled water the number of H ions is equal to the number of OH ions, each must have a concentration of  $1/10,000,000$  or  $10^{-7}$ .

**The Hydrogen-ion Exponent Scale, or pH Scale.**—In order to simplify and to emphasize the importance of hydrogen-ion concentration, Sørensen<sup>2</sup> proposed using the numerical values of the negative exponents of hydrogen-ion concentration,  $[H^+]$ , as the basis of a hydrogen-ion concentration scale and suggested the symbol  $P_{H^+}$ . Clark later proposed using the simpler symbol, pH. Clark's symbol will be used in this book. Since the pH value is the logarithm of the reciprocal of the hydrogen-ion concentration,  $[H^+]$ , the general relation may be expressed as follows:

$$pH = \log \frac{1}{[H^+]} \quad \text{or} \quad [H^+] = 10^{-pH}.$$

This method of expressing H-ion concentration has now been generally adopted. In the case of pure distilled water, in which the H-ion concentration is  $0.0000001$  or  $N/10,000,000$ , the pH value would therefore be the log of  $1/0.0000001$  or  $10,000,000$ , which is  $7.0$ . This value  $7.0$  is, therefore, the neutral point on the pH scale.

THE pH SCALE		
ACID	NEUTRAL	ALKALINE
←-----→		
etc. 5.6, 5.8 6.0, 6.2, 6.4, 6.6, 6.8,	7.0,	7.2, 7.4, 7.6, 7.8, 8.0, 8.2, 8.4, etc.

It will readily be seen that pH values below  $7.0$ , such as  $6.0$ ,  $5.0$ , etc., will denote acidity, the degree of acidity increasing as the numbers decrease. For example, if a small amount of HCl is added to pure distilled water which has an H-ion concentration of  $10^{-7}$  or a pH value of  $7.0$ , the acid will dissociate into positive H ions and negative Cl ions. The total number of H ions in the solution will, therefore, be greater than  $10^{-7}$ , and the solution will therefore be acid. Suppose the H-ion concentration is found to be  $N/1,000,000$ . The pH value will

<sup>2</sup> Biochem. Z., **21**, 131 (1909).

be the log of 1,000,000, which is 6.0. Similarly, if the H-ion concentration is  $N/100,000$ , the  $pH$  value will be 5.0, etc. It should be remembered that, since we are dealing with logarithmic values, a solution having a  $pH$  value of 6.0 contains 10 times as many H ions as one having a  $pH$  value of 7.0. Similarly, a solution of  $pH$  5.0 contains 100 times as many H ions as one of  $pH$  7.0.

Since, by the *Law of Mass Action*

$$\text{Conc. of H ions} \times \text{conc. of OH ions} = \text{a constant},$$

it is apparent that as the H-ion concentration increases the OH-ion concentration must decrease, and *vice versa*. Even in a strongly alkaline solution there are, however, some H ions and, for the sake of simplicity, it is advisable to express both acidity and alkalinity in terms of  $pH$  values.

From the fact that neutral water has an H-ion concentration of  $N/10,000,000$ , it is apparent that an alkaline solution must contain a smaller number of H ions. Let us assume that by adding a small amount of sodium hydroxide to pure water, we decrease the H-ion concentration to  $N/100,000,000$ . The  $pH$  value of this solution would be 8.0. Similarly, if the H-ion concentration is reduced to  $N/1,000,000,000$ , the  $pH$  value would be 9.0. This shows why all values higher than 7.0 indicate alkalinity, the degree of alkalinity increasing as the numbers increase. It should be remembered here also that a solution of  $pH$  9.0 contains 10 times as many OH ions as one of  $pH$  8.0.

It is therefore seen that the  $pH$  value is an absolutely accurate measure of the *degree* of acidity or alkalinity of a solution. By making these determinations, definite values are obtained which can be recorded and which can be duplicated exactly at any time by the same or different workers. We are, therefore, not dependent on such vague and meaningless terms as slightly acid or alkaline, moderately or strongly acid or alkaline, etc.

The exact meaning of H-ion concentration and  $pH$  values, and the derivation of the latter, can be made clear by a few concrete examples. An  $N/10$  solution of hydrochloric acid is one that contains 3.65 grams of HCl or 0.1 gram of *ionizable* H per liter. Electrical conductivity measurements have shown that at 18° C. 91.4 per cent of the HCl is dissociated. This means that 91.4 molecules out of every 100 do not exist as molecules, but are split up into ions of H and Cl. The remain-

ing 8.6 per cent of course exist as molecules. If the HCl were completely ionized, the N/10 solution would contain 0.1 gram of H ions per liter. Since, however, only 91.4 per cent is ionized, it contains  $0.1 \times \frac{91.4}{100} = 0.0914$  gram of H ions per liter. The normality of this

solution with respect to H ions is  $\frac{1}{0.0914} = N/10.94$ . The pH value of

N/10 hydrochloric acid is, therefore, the logarithm of 10.94, or 1.04.

An N/10 solution of acetic acid also contains 0.1 gram of *ionizable* H. Electrical conductivity measurements show, however, that at 18° C. it is dissociated only to the extent of 1.36 per cent. Hence the hydrogen-ion concentration is  $0.1 \times \frac{1.36}{100} = 0.00136$  gram per liter.

This is equivalent to an N/735 solution of H ions. The pH value of N/10 acetic acid is therefore the log of 735, which is 2.86.

Since the hydrogen-ion concentration of N/10 HCl is 0.0914 gram per liter and of the acetic acid 0.00136 gram per liter, the hydrochloric acid contains almost 70 times as many hydrogen ions as the acetic acid.

Table XV will give the worker some idea of the relationship between the total acidity and pH value of a few common acids and bases. The pH values have been given for N/10 solutions and are rounded off to the nearest 0.1, as this is sufficiently accurate for general work. Thus, the above value for acetic acid, pH 2.86, is given as 2.9 in the table.

TABLE XV

Acids	pH Value	Bases	pH Value
HCl	1.0	C <sub>6</sub> H <sub>5</sub> NH <sub>2</sub>	7.8
H <sub>3</sub> PO <sub>4</sub>	1.5	NaHCO <sub>3</sub>	8.4
CH <sub>3</sub> COOH	2.9	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub>	9.2
H <sub>2</sub> CO <sub>3</sub>	3.8	NH <sub>4</sub> OH	11.3
H <sub>3</sub> BO <sub>3</sub>	5.2	Na <sub>2</sub> CO <sub>3</sub>	11.6
C <sub>6</sub> H <sub>5</sub> OH	6.0	NaOH	13.1

**Buffer Action.**—It is clear from the above discussion that, if the solutions which are encountered in chemical processes, bacteriological and pathological work, etc., were only solutions of known pure acids and alkalies, the pH value could be calculated from the titration



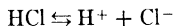
values and ionization constants. This is, however, seldom the case, as the solutions normally contain relatively indefinite quantities of other substances, and usually a number of unknown impurities. Many, and in fact most, of these materials have what is known as "buffer action," which is described by Clark as the resistance exhibited by a solution to change in  $pH$  through the addition or loss of acid or alkali. This action can best be illustrated by means of an example. Pure water, as stated above, has a  $pH$  value of 7.0. If 1 cc. of 0.01 N HCl is added to a liter of pure water, the  $pH$  value will be changed to about 5.0. Let us now consider a solution containing a mixture of sodium acetate and acetic acid. In this solution the dissociation of the acetic acid is very small:



The dissociation of sodium acetate is, however, large:



Now suppose we add a small quantity of HCl to this solution. It is immediately largely dissociated into H and Cl ions:



We therefore have in solution H, Cl, Na and  $\text{CH}_3\text{COO}$  ions. Acetic acid ionizes to only a very slight degree. This means that  $\text{CH}_3\text{COO}$  and H ions cannot exist together in solution to a very large extent. Therefore, the larger part of the H ions from the HCl will immediately combine with  $\text{CH}_3\text{COO}$  ions to form undissociated molecules of acetic acid, and the H ion concentration or  $pH$  value of the solution will be only very slightly changed, if at all. If, on the other hand, NaOH is added to this mixture, it dissociates into Na and OH ions:



The OH ions' then react with the H ions from the acetic acid to form water. More acetic acid will then dissociate into  $\text{CH}_3\text{COO}$  and H ions and the H-ion concentration will be practically the same as it was before. It will, therefore, be seen that considerable quantities of acids or alkalis may be added to solutions containing buffer salts without changing the  $pH$  value. Since this is true, it is clear that buffered solutions can be diluted with distilled water, even though the water shows a very acid reaction, without affecting the  $pH$  value. In

fact, some solutions can be diluted as much as 1000 to 1. This is of importance in making determinations on very highly colored and turbid solutions.

In general, the salt of any weak acid or weak base is a buffer salt. There are, therefore, very few solutions which are free from buffer action. For example, the phosphates in raw sugar and culture media, carbonates in raw water, alum and rosin in paper sizing, etc., have buffer action.

In order to bring out the importance of making  $pH$  determinations rather than determining the total acidity or alkalinity by titration, we shall consider a solution of raw sugar, which contains phosphates as well as other buffer compounds. By the addition of small amounts of acid or alkaline materials, the total acidity or alkalinity is increased, and this additional acid or alkali is shown by titration. Since the solution is highly buffered, however, there will be practically no change in the  $pH$  value. Since it is the  $pH$  of the solution, that is, the amount of ionized acid, which determines the amount of inversion of cane sugar, the reason for determining the  $pH$  rather than the total acidity is apparent. The figures given below are actual determinations which were made on liquors in a sugar refinery:

Per Cent Acidity by Titration	$pH$ Values	Per Cent Alkalinity by Titration	$pH$ Values
0.001	6.5	0.001	8.3
0.010	6.4	0.003	7.3
0.005	5.3		
0.005	7.0		

In the first pair of figures, the acidity of the second liquor is just 10 times that of the first one, as determined by titration. The  $pH$  values of the two are, however, practically the same. In the second pair of figures both have an acidity by titration of 0.005 per cent, while the  $pH$  values show the acidity of the first to be much higher (in fact almost 100 times greater) than the second. The third pair of figures shows even greater discrepancy. By titration the second solution is 3 times as alkaline as the first. By  $pH$  measurements, however, the first is much more alkaline (10 times) than the second. While these figures represent measurements on sugar liquors, solutions from other processes show similar discrepancies.

**Unbuffered Solutions.**—From the above discussion it will be realized that no special precautions are necessary in making  $pH$  measurements on buffered solutions, since the addition of considerable amounts of acid or alkali can be made without affecting their  $pH$  value.

Such is not, however, the case with distilled water or with unbuffered solutions, especially when their  $pH$  value is near the neutral point of 7.0. The fact that distilled water is one of the most difficult materials to test for  $pH$  is frequently overlooked. This is due to the fact that it is absolutely devoid of any buffering action, and is thus very susceptible to change during the test, for example, by the absorption of carbon dioxide, etc.

Pure distilled water is, of course, free from salts and has a  $pH$  value of 7.0 at 22° C. However, the reaction of ordinary distilled water is always acid, because of the absorption of carbon dioxide. Water which has taken up carbon dioxide from the air until equilibrium has been established will contain about 0.3 per cent of  $CO_2$  by volume, and the calculated  $pH$  should be 5.7. In fact, this is the value which is usually found in distilled water which has been freely exposed to pure air. Water from an efficient automatic still, when stored in closed non-soluble glass vessels, will have a  $pH$  of 6.0 to 6.4. If this water is boiled for a short time in a Pyrex vessel, and the vessel then fitted with a soda lime tube, it will usually have a  $pH$  of 6.6 to 6.8.

When it is necessary to make a solution of an unbuffered material in order to determine its  $pH$  value, the water used should of course be as nearly neutral as possible, as any acidity of the water will affect the  $pH$  of the dissolved material. Water having a  $pH$  value of 6.6 to 6.8 is satisfactory for this work. In making these solutions, the proportion of material to water should always be kept the same, so that different determinations will be comparable. It is of course equally true that the indicator solutions should have a neutral reaction, since any excess acid or alkali will likewise change the  $pH$  value of the material.

These precautions are particularly important when the  $pH$  value of the material is near 7.0. Around this point small variations in hydrogen-ion concentration, due to absorption of carbon dioxide, etc., have a marked effect on the  $pH$  value. This effect is of course much less marked when we get below 6.0 or above 8.0.

In making a test on distilled water or unbuffered solutions, the indicator and the material being tested should always be mixed in the

test tube by means of a stirring rod, with the minimum of exposure to air, and readings should be made at once. If this is done, reliable results will be secured with the colorimetric method. In fact, this is the only way in which such solutions can be tested for  $pH$ , since electrometric methods are very unreliable when applied to most unbuffered solutions.

#### MAKING HYDROGEN-ION DETERMINATIONS

**Indicators.**—The principle of making  $pH$  measurements is based on the fact that various indicators change in color when they are acted upon by solutions of different acidities or alkalinities. Litmus paper is probably the simplest and best known example of an indicator. Its color change is from red to blue. That is, if litmus paper is dipped into an acid solution, it turns red. If it turns only slightly red, we say the solution is "slightly" acid. If it turns deep red, however, we say the solution is "strongly" acid. Similarly, if the paper turns blue, we say the solution is "slightly" or "strongly" alkaline. These are very vague and indefinite terms and their meaning varies enormously, depending largely on the personal opinion of the worker. Thus, what is "slightly" acid to one worker might be considered as "strongly" acid by another, and vice versa.

In making  $pH$  measurements, we simply substitute definite  $pH$  values, such as 3.0, 6.0, 6.8, 7.4, 9.6, etc., for the indefinite terms "strongly" and "slightly" acid and alkaline. These  $pH$  values can be recorded and can be duplicated at any time by the same or different workers.

Theoretically, litmus and other test papers can be used for determining the  $pH$  of a solution. In practical application, however, the error is too great. One of the chief reasons for the inaccuracy of litmus is that the range is so wide ( $pH$  4.6 to 8.4) that the color changes are not distinct. It is practically impossible to tell the difference in the colors produced by solutions having  $pH$  values which differ by 1.0  $pH$  unit. In fact it has been shown in actual practice that solutions which specially prepared litmus indicated to be alkaline were really acid and vice versa, when accurate  $pH$  measurements were made on the same solutions.

Some other test papers are more sensitive than litmus, but it is very doubtful whether differences of less than 0.6  $pH$  can be detected by their use. Even this degree of accuracy is seldom obtainable, as it

is very difficult to obtain two batches of test paper which will give the same readings. Granting that a uniform paper can be obtained, we have no standards by which to judge. Thus a shade which would be considered as corresponding to a  $pH$  value of 6.0 by one worker, might be considered as 6.6 by another. Since variations as large as 0.2  $pH$  may be of serious consequence, the need for an accurate method is apparent.

As stated above, litmus is very insensitive, partly because it covers such a wide range. In order to make accurate  $pH$  determinations it is, therefore, necessary to employ indicators with short ranges, so that the color changes are more distinct. This means that we need a number of indicators to cover the entire  $pH$  range 1.2 to 13.6. A complete set of indicators, which were developed for just this purpose, are given, with their respective  $pH$  ranges and color changes, in Table XVI. This table should be studied carefully so that the worker is thoroughly familiar with the various indicators, for with this knowledge he will have no difficulty in understanding the following discussion.

TABLE XVI

Name	$pH$ Range	Color Change
Metacresol purple.....	1.2 - 2.8	Red - Yellow
LaMotte yellow.....	2.6 - 4.2	Red - Yellow
Bromphenol blue.....	3.0 - 4.6	Yellow - Blue
Bromcresol green.....	4.0 - 5.6	Yellow - Blue
Chlorphenol red.....	5.2 - 6.8	Yellow - Red
Bromthymol blue.....	6.0 - 7.6	Yellow - Blue
Phenol red.....	6.8 - 8.4	Yellow - Red
Cresol red.....	7.2 - 8.8	Yellow - Red
Thymol blue.....	8.0 - 9.6	Yellow - Blue
Nitro yellow.....	10.0 - 11.6	Colorless - Yellow
Sulfo orange.....	10.0 - 12.6	Yellow - Orange
LaMotte violet.....	12.0 - 13.6	Red - Blue

The color changes of these indicators are similar to that of litmus. For example, litmus is red in an acid solution ( $pH$  4.6) and blue in an alkaline solution ( $pH$  8.4). Similarly, chlorphenol red has a yellow color at  $pH$  5.2 and a deep red color at  $pH$  6.8; bromthymol blue is yellow at  $pH$  6.0 and deep blue at  $pH$  7.6, etc.

**Rough Tests.**—If the worker has no idea of the  $pH$  value of a given solution, it is necessary first to make a rough determination. To make the test, fill three or four graduated test tubes to the mark

(10 cc.) with the solution to be tested. To the first one add 0.5 cc. of bromthymol blue indicator solution. This indicator is tried first since it has a  $pH$  range of 6.0–7.6, and, therefore, covers the neutral point,  $pH$  7.0. Hence it is possible by means of this one indicator to determine whether the solution being tested is neutral, acid, or alkaline. The color change of bromthymol blue is from yellow at 6.0 to a deep blue at 7.6. Therefore, if on adding this indicator solution, a color intermediate between yellow and deep blue is obtained, the  $pH$  of the solution lies between 6.0 and 7.6, and it is either neutral or very slightly acid or alkaline. In this case no further rough tests are necessary.

If, however, a yellow color is obtained on adding the bromthymol blue indicator solution, the  $pH$  of the solution is at least 6.0 and possibly lower. This is due to the fact that  $pH$  6.0 is the acid end of the range of this indicator. Even if the solution had a  $pH$  of 5.0 or 4.0, it would still give a yellow color with bromthymol blue. If then a yellow color is obtained, 0.5 cc. of the bromcresol green indicator solution, which, it will be seen by reference to the table, covers a more acid part of the range ( $pH$  4.0–5.6), should be added to the second test tube containing the solution of unknown  $pH$ . The color change for this indicator is from yellow at  $pH$  4.0 to a deep blue at  $pH$  5.6. If, therefore, a color intermediate between yellow and deep blue is obtained in this test, the  $pH$  of the solution lies between 4.0 and 5.6.

These two indicators do not cover quite all the values between 4.0 and 7.6. Thus, if bromcresol green ( $pH$  4.0–5.6) gives a deep blue color and bromthymol blue ( $pH$  6.0–7.6) a yellow color, it is apparent that the  $pH$  of the solution lies around  $pH$  5.6–6.0, that is, within the range of chlorphenol red ( $pH$  5.2–6.8). Of course, only one indicator should be added to each tube. That is, if bromthymol blue gives a yellow color, the bromcresol green must always be added to a fresh tube. If a yellow color is obtained with bromcresol green, the test should be repeated, using bromphenol blue which covers a still more acid part of the range,  $pH$  3.0–4.6, etc.

If, on the other hand, on adding bromthymol blue indicator solution in the first test, a deep blue color was obtained, the solution is alkaline and has a  $pH$  value of at least 7.6 and possibly higher. The test should then be repeated using thymol blue, which covers a more alkaline part of the range,  $pH$  8.0–9.6.

This procedure must, of course, be followed in determining the range required for any specific work, that is, a rough test must be made

on solutions from all steps of the process, in order to determine what equipment should be purchased.

**Accurate Measurements.**—Let us assume that in the first rough test, that is, the one in which bromthymol blue indicator solution was added to the solution in the test tube, a color intermediate between yellow and deep blue was obtained. This would show that the  $pH$  of the solution lies between 6.0 and 7.6. It is now a question of determining the exact  $pH$  value. For this purpose a set of bromthymol blue color standards and some form of comparator are required. A set of Bromthymol Blue Color Standards consists of 9 standard tubes and one tube of distilled water, as shown in Fig. 43.

The color standards for bromthymol blue are simply 9 tubes, containing 10 cc. of solutions of definite  $pH$  value, that is 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4 and 7.6, to each of which 0.5 cc. of bromthymol blue indicator solution has been added. It is seen that these standards are made up in exactly the same manner in which the test was made, except that solutions of definite  $pH$  values were used. These color standards can be purchased and it is, therefore, not necessary or advisable for the worker to make them up for himself.

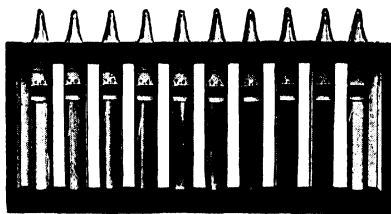


FIG. 43.—Bromthymol Blue Color Standards.

The 9 tubes are labeled with the  $pH$  values of the various solutions which they contain. The tube marked  $pH$  6.0 will have a yellow color and that marked  $pH$  7.6 a deep blue color. The intermediate tubes, which are marked 6.2, 6.4, 6.6, etc., will have colors intermediate between yellow and blue.

All that is now necessary is to match the test sample with the standards. When a match is obtained, the  $pH$  value of the solution is read off directly from the standard with which it matches.

If the rough test had shown the  $pH$  of the solution to lie between 5.2 and 6.8, a tube of the unknown, to which chlorphenol red indicator solution has been added, must of course be compared with a set of chlorphenol red color standards. These are similar to the bromthymol blue standards. Thus the tube marked  $pH$  5.2 has a yellow

color and that marked  $pH$  6.8 has a deep red color. The intermediate tubes have colors intermediate between yellow and red. Analogously, if the  $pH$  of the solution were found to be between 4.0 and 5.6, the tube to which the bromcresol green indicator solution was added must be compared with the bromcresol green color standards, etc.

It will be seen above that the ranges of the various indicators overlap. That is, bromthymol blue covers the range  $pH$  6.0 to 7.6 and chlorphenol red the range  $pH$  5.2 to 6.8. The values 6.0 to 6.8 are thus common to both indicators. It is, therefore, clear that if the  $pH$  value of the solution which is being tested lies between 6.0 and 6.8, determinations can be made with both indicators. That is, the sample to which the bromthymol blue indicator solution is added is compared with the bromthymol blue color standards, and the one to which the chlorphenol red is added is compared with the chlorphenol red color standards. As almost all of the indicators overlap, it is usually possible to make determinations with two indicators and thus check results.

The worker should, however, be warned against possible errors due to matching against the color standard on either end of any given set. Thus, a test sample may match the bromthymol blue standard  $pH$  7.6 and yet have a much higher  $pH$  value, due to the fact that  $pH$  7.6 is to the end of the range of bromthymol blue. In such a case the test should be repeated using phenol red.

After a few actual determinations have been made the above procedure will be found to be very quick and simple. In fact the worker will quickly learn by experience which indicator should be used in each step of a process and can therefore make the comparison directly, without first making the rough test.

Then, too, it is not always necessary to determine the exact  $pH$  of a solution. For example, suppose a solution is to be kept at a  $pH$  of 7.8. By adding 0.5 cc. of phenol red indicator solution to a 10 cc. sample in one of the graduated test tubes, and comparing it with the phenol red color standard,  $pH$  7.8, it can be determined whether the solution is at the correct point,  $pH$  7.8, or whether the  $pH$  is too high or too low. If the color obtained in the test sample is a lighter red than the 7.8 standard, the  $pH$  is too low. Therefore alkali must be added until another test gives a match with the 7.8 standard. If, on the other hand, the color obtained is a darker red than the 7.8 standard, the  $pH$  is too high, and acid must be added to bring the solution back to the desired  $pH$ .



**Procedure for using the LaMotte Block Comparator.**—It is first necessary to make a rough determination of the  $pH$  of the solution to be tested, by the procedure given above. Let us assume that this test has shown the  $pH$  of the solution to lie within the range of bromthymol blue,  $pH$  6.0–7.6. A Block



FIG. 44.

Comparator, Fig. 44, containing bromthymol blue color standards would therefore be required to make an accurate measurement.

First, remove the top of the case and use it as a comparator block. The six holes back of the slots in the side of this block will be designated as *B*, *A*, *C* and *E*, *D*, *F* respectively, as shown in Fig. 45.

Fill three of the graduated test tubes to the mark (10 cc.) with the liquid to be tested and place them in the holes marked *B*, *A* and *C*. To the middle tube (*A*) add 0.5 cc. of bromthymol blue indicator solution from the bottle, by means of the graduated pipette and nipple, and shake the contents thoroughly. Place the tube of distilled water in the hole marked *D*, and two of the color standards, differing by only 0.2  $pH$ , for example 6.8 and 7.0, in the two holes *E* and *F*. Look through the three pairs of tubes, holding them toward the light, and changing the color standards if necessary, until the central pair of tubes exactly matches either of the other pairs, or until the color through the central pair lies between the colors of the pairs on either side. Always make

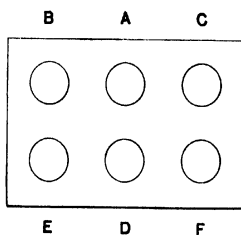


FIG. 45.

sure that the standards placed on either side of the ampoule of distilled water differ by only 0.2  $pH$ , that is, 6.8 and 7.0, and 7.2, etc. If an exact match is obtained, the  $pH$  of the solution is read off directly from the standard with which the match is obtained. If, however, the color of the central pair of tubes lies between the colors of the pairs on either side, the  $pH$  value is taken as the average of the two. For

example, if it lies between 6.8 and 7.0, the value is taken as 6.9. The piece of etched glass which is placed over the slots on one side of the block is a great aid in making accurate measurements, as it eliminates the reflection of outside objects in the tubes. The block should be held so that the etched glass is on the side facing the source of light.

If it had been found, by the first rough test, that the  $pH$  of the solution lay between  $pH$  5.2 and 6.8, the determination would of course be made in an exactly similar manner, using a Block Comparator containing chlorophenol red color standards and indicator solution.

As has been shown on page 209, since the  $pH$  ranges of practically all of the indicators overlap, it is usually possible to make determinations on a given sample with two different indicators and thus check the results obtained.

The reason for the special arrangement of the tubes in the comparator block, as illustrated in Fig. 45, is to eliminate any effects of color or turbidity in the sample which is being tested. For example, suppose that we are testing a green solution. We shall designate this green solution by  $G$ , water by  $W$  and the indicator solution by  $I$ . Referring to Fig. 46, the contents of the test tubes and color standards are represented as follows, the various tubes being indicated by the same letters which are used in Fig. 45.

To make this clear, the color standards may be considered as made up of water and indicator solution. They do of course contain solutions of salts to give them definite  $pH$  values, as described under "Color Standards," but, since the solutions of salts from which they are made are clear and colorless, they may be considered as made up of only these two materials.

It will be seen from Fig. 46 that each pair of tubes ( $B$  and  $E$ ,  $A$  and  $D$ ,  $C$  and  $F$ ) contains the green solution  $G$ , indicator solution  $I$  and

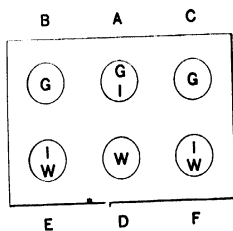


FIG. 46.

water  $W$ , the only difference being that the indicator solution has been added to the green solution in the central test tube, instead of to the distilled water tube which is placed in front of it. The final combination is, however, the same in all pairs. It is therefore apparent that, when the proper color standards have been inserted, an exact match

must be obtained, since the observer is looking through exactly the same materials in each pair of tubes.

It is also apparent that, in making the first rough tests to determine the approximate  $pH$  value, the color or turbidity of the solution may obscure the true color of the indicator. Thus, in testing the green solutions of nickel salts with bromcresol purple, which is the indicator that is used in regulating the acidity of nickel electroplating baths, the green color of the solution, of course, affects the color of the bromcresol purple indicator solution. In the first tests which are made it is, therefore, sometimes necessary to make an actual determination with the color standards in order to determine which indicator covers the range of the solution which is being tested. After a few measurements, however, the worker learns to recognize the characteristic colors which are given by the various indicators in his specific solution, and can easily tell the standards with which comparison should be made.

Unless the solution is so dark or turbid that light will not pass through it, the  $pH$  can be determined directly by means of the Block Comparators. Most solutions which are so highly colored or turbid are, however, highly buffered, which means that they can be diluted without changing their  $pH$  value. In this way the color or turbidity can be decreased to such an extent that accurate readings can be made. In fact, color and turbidity can often be practically eliminated.

Since solutions of this sort are met with from time to time, it is worth while to outline a procedure for determining how far a given solution can be safely diluted. As a rough test, place 20 cc. of the material to be tested in each of four beakers. To the first add 20 cc. of distilled water, to the second 50 cc., to the third 100 cc., and to the fourth 400 cc. Mix each thoroughly and determine the  $pH$  value of each. If all give the same value, dilution to this extent, that is, 20 to 400 or 1 to 20, is allowable. In this case even greater dilutions may be tried, as some materials may be diluted as much as 1 to 1000 without changing the  $pH$  to any appreciable extent.

If, however, it is found that dilutions of 20 to 20, 20 to 50 and 20 to 100 give the same  $pH$  value, but the dilution 20 to 400 gives a different value, a few more mixtures should be made, such as 20 to 200 and 20 to 300, in order to determine the largest allowable dilution. After this point is once determined for a given material this dilution can be used in future determinations. It is, however, safest to adopt as standard a dilution which is somewhat below the upper limit.

This same principle holds true in making determinations on solid materials. Such materials should be broken up or ground as finely as possible, and then dissolved or suspended in varying amounts of distilled water, for example 1 to 2, 1 to 5, 1 to 10, 1 to 25, 1 to 50, etc. After allowing sufficient time for complete solution or extraction,  $pH$  determinations should be made and the results interpreted as outlined above for colored and turbid solutions.

Although the  $pH$  of an unbuffered solution does change with dilution, by always using the same proportions of the material and distilled water, for example, 1 to 10 or 1 to 20, a working value may be obtained, which is just as satisfactory for control work as if it were the real  $pH$  of the material. In such cases the distilled water used should, of course, have the same  $pH$  value in all determinations. The precautions to be observed in making measurements on unbuffered solutions have been given on page 204.

The Block Comparator is recommended for use where it is desired to cover only a short range, for example, that of one indicator, such as bromthymol blue. It is also useful for field work where it is necessary to cover a wider range.

Three or four Block Comparators can easily be carried in a hand bag and furnish all the equipment necessary for making tests. More convenient equipment is available for work which requires the use of more than one indicator

**LaMotte Hydrogen-Ion Testing Set.<sup>3</sup>—Figure 47.**

The development of this complete, portable outfit for the colorimetric determination of hydrogen-ion concentration, according to the method of Brown,<sup>4</sup> has revolu-



FIG. 47.—LaMotte Hydrogen-Ion Testing Set (Model 5B). A single test can be made in a few seconds. Only one drop of material is needed for a test. Results are accurate to 0.1  $pH$ .

<sup>3</sup> These sets are made in three sizes. They may be obtained from the LaMotte Chemical Products Co., Baltimore, Md.

<sup>4</sup> J. H. Brown, *J. Lab. Clin. Med.*, 9, 239 (1924).

tionized  $pH$  determinations in general bacteriological, biological and pathological, as well as general research and industrial work. Although this set was designed primarily for use where only small amounts are available for the test, its many advantages recommend its use in innumerable other cases, even though the material to be tested is plentiful. Actual practice has shown this method to be ideally suited for work with culture media, serums, urine, feces, gastric and duodenal contents, muscle juice, tissue extracts, milk, fruit juices, all other plant and animal secretions and extracts, clays, and innumerable other materials.

Recent work has demonstrated the special advantages of this equipment for sewage work, the testing of milk, control in the manufacture of milk products, such as acidophilus and buttermilk, cheese, ice cream, etc.

**Procedure.**—It is, of course, first necessary to determine the indicator whose range covers the  $pH$  value of the sample which is being tested. The procedure is exactly similar to that given above, except that the glass cells are used instead of test tubes. For example, place several of the cells on the plate and fill them with the material which it is desired to test. Add a drop of the bromthymol blue indicator solution to the first cell by means of the pipette and nipple. This will tell whether the material is neutral, acid, or alkaline. If it proves to be as acid as  $pH$  6.0, that is, if a yellow color is obtained with bromthymol blue, add a drop of methyl red to the second cell, etc., until the proper indicator is determined.

Let us assume that the  $pH$  of the sample being tested is within the range of chlorphenol red, that is, between  $pH$  5.2 and 6.8. Nine of the small glass cells, shown in the vials, in the right-hand side of the case, are placed on the opal glass plate, directly under the 9 etched numbers covering the range of chlorphenol red, 5.2–6.8. These cells are then filled with the corresponding buffer mixtures from the etched vials, by means of the pipettes and nipples. One drop of the chlorphenol red indicator solution is then added to each of these cells of buffer mixture, care being taken to hold the pipettes vertically, so that drops of the same size are placed in each cell. Since the indicators are in alcoholic solution, instant mixing takes place. This set of 9 cells then constitutes the color standards, and it will be seen that they are similar to the color standards which are used with the Block Comparator, except that they are made up in cells instead of ampoules. When once set up,

the color standards in the cells may be used for all determinations made during the day; the cells must however, be kept level full by adding a drop of distilled water from the vial from time to time, to replace that lost by evaporation.

The material to be tested is then placed in another cell, below the row of standards, and a drop of the same indicator solution added. By sliding this cell along the plate, by means of the tweezers or the platinum loop, its color can be matched with the proper standard cell and the pH value read off directly. Measurements made with these sets have been shown to check with extreme accuracy those made on the same solutions by means of the hydrogen electrode.

All cells should be filled so that the surface is level, that is, neither concave nor convex, since this eliminates shadows and makes the color uniform throughout.

It is apparent that unless the material being tested is practically clear and colorless, an exact match with the standards cannot be obtained, as the color or turbidity of the unknown would affect the color of the indicator when it is added. It is fortunately true, however, that practically all of the materials in which bacteriologists, pathologists, biologists, etc., are interested, are highly buffered. It is therefore clear that the pH would not be changed by dilution with distilled water.

This makes it possible to make a determination with an extremely small amount of material. For example, a drop from one of the sampling pipettes or a loopful of the material is placed in one of the small cells, and the cell is then filled with distilled water from the vial. Dilution to this extent does not change the pH value, but does eliminate any effects of color or turbidity, so that an accurate match can be obtained.

The degree to which a material can be diluted has been discussed under the "Block Comparator," page 212, and it is advisable for those who are not familiar with this matter to read the discussion given there.

In case the material to be tested is not highly buffered, as with water, the cell should be filled with the material. If, however, the unbuffered material is highly colored or turbid, the Block Comparator should be used for making determinations.

The fact that the ranges of the indicators overlap, as shown by Table XVI on page 206, makes it possible to check determinations with

two indicators. Thus, if a material which is being tested has a  $pH$  value between 6.0 and 6.8, it can be measured with both chlorphenol red ( $pH$  5.2–6.8) and bromthymol blue ( $pH$  6.0–7.6), etc. This gives excellent facilities for proving the accuracy of the method.

### Some Applications of Hydrogen-ion Control.

1. Acid zinc plating.
2. Bacteriological work.
3. Candy.
4. Canning of food products.

For detailed data on the  $pH$  value of various food products and other valuable information, see Bulletin No. 17-L. Research Laboratory, National Canners' Association.

5. Chemical analysis.

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16. Electroplating.
17. Paper manufacture.
18. Pathological work.
19. Pharmaceuticals.
20. Sewage disposals.

For detailed information, see Proceedings of the Eleventh Annual Meeting of the New Jersey Sewage Works Association, 1926, and Wilson, *J. Ind. Eng. Chem.*, **13**, 406 (1921); *ibid.*, **14**, 128 (1922); *ibid.*, **15**, 956 (1923).

21. Sugar manufacture and refining.

For detailed information on the importance of  $pH$  control in sugar manufacture and refining, see the excellent article of Blowski and Holven, *Ind. Eng. Chem.*, **17**, 1263 (1925); or *The Planter* for March 6, 1926, p. 188.

22. Tanning of leather.
23. Water purification.
24. Cleaning and laundry processes.



## CHAPTER XX

### IRON

#### DETERMINATION OF IRON AS THE SULFOCYANATE (THIOCYANATE)

##### METHOD OF STOKES AND CAIN<sup>1</sup>

THIS method is based upon the formation of ferric sulfocyanate when a sulfocyanate solution is added to a solution containing ferric ions. The intensity of the color of ferric sulfocyanate, although very great, is extremely dependent upon the *composition* of the solution and is by no means proportional to the concentration. The red color is due to the undissociated salt and to its double compounds, the ionized salt being colorless. The salt is, further, very prone to hydrolysis. Many substances interfere markedly with the reaction, notably fluorides, phosphates, arsenates, oxalates, citrates, tartrates, iodates, and to a less but still marked degree acetates and sulfates, the action of some of these being so strong that it is impossible to get the color even with considerable quantities of iron. In short, the intensity of the color is so influenced by the nature and concentration of the substances present that unless the test solution and the standard solution with which it is compared have identical composition and concentration, results varying many hundred or even thousand per cent from the truth may be obtained.

Thompson<sup>2</sup> was the first to propose the direct determination of iron by the color of the sulfocyanate in aqueous solution, but this method can only be used when it is possible to have the sample and standard solutions identical. Tatlock<sup>3</sup> greatly improved the method by extracting the ferric sulfocyanate with ether and comparing the

<sup>1</sup> H. N. Stokes and J. R. Cain, J. Am. Chem. Soc., **29**, 409 (1907); *ibid.*, **29**, 443. In writing this method the author was aided by Mr. J. R. Cain, who very kindly furnished an outline of what to reproduce from the work of Stokes and Cain.

<sup>2</sup> J. Chem. Soc., **47**, 493 (1885).

<sup>3</sup> J. Soc. Chem. Ind., **6**, 276, 352 (1887), based on Natanson's [Ann. **130**, 246 (1864)] observation that the reaction is more sensitive when ether is used as solvent.

color with ethereal layers of the same volume and thickness containing known amounts of iron. The extraction, however, is never complete, and the less so, the more interfering substances are present. The method was improved and given a somewhat wider scope by Lunge and von Keler,<sup>4</sup> but there are a number of features of their method which are not satisfactory.

Stokes and Cain attribute the abnormal behavior of the color of the ether solution to the presence of peroxides. The color of the solution in ether that has been freed from peroxides by shaking with ferrous sulfate solution is pure pink or rose from the start; if, however, a drop of hydrogen peroxide be added, the solution becomes a dirty yellowish pink, which becomes pure after a time, with deposition of a yellowish solid between the two layers. This is probably pseudosulfocyanogen, which is formed by the action of the peroxide in the isodisulfocyanic acid which is present. (Note 1.)

The fact that ether gave discolored solutions led Stokes and Cain to try other solvents. Of these, amyl alcohol proved to be the most satisfactory, giving a perfectly pure color from the start, and being a decidedly better solvent than ether for ferric sulfocyanate. A rather crude experiment with an intensely colored aqueous solution of ferric sulfocyanate, with excess of ammonium sulfocyanate and hydrochloric acid showed that ether left 3.7 times as much iron as was left by an equal volume of amyl alcohol. The relative efficiency doubtless varies with the composition of the solution, but the above figures show the decided superiority of amyl alcohol. Although Stokes and Cain later discovered a method of inhibiting the discoloring action of peroxides on sulfocyanates, they retained the use of amyl alcohol on account of its better solvent action, mixing it, however, with a certain proportion of ether. Amyl alcohol is somewhat too viscous to allow rapid separation. When mercuric sulfocyanate reagent is used, ether is a relatively still poorer solvent for ferric sulfocyanate and under certain conditions has the unusual property of forming three layers, the iron being mostly concentrated in the middle layer. These objectionable features are entirely obviated by using a mixture of 5 volumes amyl alcohol with 2 volumes ether, and it is this mixture which is meant whenever the amylic layer is spoken of below.

Stokes and Cain have shown that traces of iron cannot be accu-

<sup>4</sup> *Z. angew. Chem.*, **1894**, 670; **1896**, 3; Lunge, *Chem. techn. Untersuchungs-methoden* 5te Aufl., **1**, 385.

rately determined in the presence of large quantities of salts and have worked out methods of concentrating the iron into a small bulk, practically free from interfering substances. These methods are described below. They also replaced ammonium sulfocyanate by free sulfocyanic acid, which may easily be prepared iron-free in a few minutes, and which serves as a solvent for the concentrated iron solution. This gives a solution of iron in a great excess of free sulfocyanic acid, practically free from other substances, and thus in the most favorable condition for the complete conversion of the iron into undissociated ferric sulfocyanate. In order to prevent the gradual bleaching of the solution of ferric sulfocyanate and to avoid the necessity of previously oxidizing ferrous iron, a few milligrams of pure potassium persulfate are added to sample and standard solution. The persulfate also oxidizes sulfocyanic acid but this action is totally inhibited by adding to the acid enough mercuric sulfocyanate to form the double salt,  $\text{Hg(SCN)}_2 \cdot 2\text{HSCN}$ . (Note 2.)

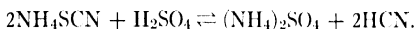
#### Reagents.

1. Standard iron solution. 0.863 gram ferric ammonium alum and 5 cc. concentrated sulfuric acid are dissolved to 1 liter. The solution, which contains 0.1 gram of iron per liter, may be kept indefinitely. For use 5 cc. are diluted to 100 cc., giving a solution of which 1 cc. contains 0.005 mg. iron. As the dilute solution hydrolyzes and deposits iron on the glass, it should be prepared fresh every day, and it is important that the measuring flask and burettes should be washed out with hydrochloric acid before using. Since persulfate is used in the cylinders the standard solution may equally well be made with the equivalent amount of ferrous ammonium sulfate, 0.702 gram per liter.

2. Mercuric sulfocyanate. The commercial article which is prepared from the nitrate and which comes as a white or yellowish powder is not to be depended upon. It is better to prepare it by pouring a hot solution of the purest mercuric chloride (1 mole) into a solution of the purest ammonium sulfocyanate (1 mole). On cooling, the sulfocyanate separates out in the form of needles, which are washed with cold water. In this case it is necessary to use twice the theoretical amount of mercuric chloride, otherwise no crystals are obtained. The excess of mercury is easily recovered by precipitation with aluminum scraps. The product is not entirely free from the double chlorine compound, but this exerts no prejudicial effect. While the yield from the nitrate is

better it contains some nitrate, which is undesirable because of its oxidizing action on sulfocyanic acid and, moreover, the nitrate is not as easily obtained free from iron.

3. Sulfocyanic acid. One hundred parts coarsely powdered ammonium sulfocyanate, which need not be free from iron, are dissolved in a stoppered graduated cylinder in a cool mixture of 65 parts by weight of concentrated sulfuric acid with 100 parts water. These correspond approximately to the equation



There is a marked fall of temperature. After the volume has been noted, the solution is transferred without delay to a separating funnel and shaken out once with three-fourths its volume of amyl alcohol. We here notice the peculiar phenomenon that iron, if present, remains entirely in the acid layer, which is usually colored pink, while the amylic layer is colorless. In order to obtain this result, however, it is necessary to adhere to the proportions given. If a more concentrated acid is used, there is considerable decomposition of the sulfocyanic acid, while, if as much as an equal volume of amyl alcohol be employed, some of the iron passes over into the latter. The amylic solution, which is found by titration with silver nitrate to contain 20–21 per cent sulfocyanic acid, is unstable and soon becomes yellow, with ultimate deposition of isopersulfocyanic acid. It is, therefore, at once shaken out twice with an equal volume of water. Sulfocyanic acid distributes itself about equally between water and amyl alcohol; the first aqueous extract, therefore, contains about 10 per cent, and the second 5 per cent, or the united extracts about 7.5 per cent, while the amyl alcohol retains about 5 per cent, in which concentration it is relatively stable and may be kept or used for making a weaker aqueous acid; it gradually turns yellow from formation of isopersulfocyanic acid, but this is entirely retained on extraction with water.

4. Sulfocyanic acid reagent. Seven per cent aqueous sulfocyanic acid freshly prepared as directed above is at once saturated with mercuric sulfocyanate, somewhat more of the latter than is required to form the compound  $\text{Hg}(\text{SCN})_2 \cdot 2\text{HSCN}$  being used, and the excess being left in the bottle. If treated with a small quantity of potassium persulfate the reagent should not impart the least yellow color to amyl alcohol, even after several hours. A slight trace of iron is occasionally observed, which comes from an impure mercuric salt. Small

amounts of this are of no significance in quantitative tests, as equal quantities of the reagent are used in each cylinder. The reagent appears to keep indefinitely, but in hot weather it is well to keep it in a cool dark place when not constantly in use.

5. Potassium persulfate. This is easily obtained free from iron by a single recrystallization, the hot concentrated solution being filtered and the crystals washed with a little cold water and carefully protected from dust.

6. Amyl alcohol and ether. A good grade of iso-amyl alcohol, such as that sold by Kahlbaum, is sufficiently pure; it need not be free from pyridine. Five volumes are mixed with 2 volumes of good ether (such as Kahlbaum's 0.720). Since the discoloring action of the peroxides on sulfocyanic acid is entirely prevented by the use of mercuric sulfocyanate, no special purification is necessary.

**Apparatus and Reagents Used in Concentrating.**—Only the best ashless filters should be used, and as even these often contain very appreciable quantities of iron they must be moistened in the funnel with 1 : 1 hydrochloric acid, allowed to stand at least fifteen minutes, and then washed with water to which a few drops of ammonia are finally added. Only Pyrex, Jena, Nonsol, or silica beakers or dishes, or platinum dishes should be used. For operations requiring long heating, or when sodium hydroxide is used, platinum is employed. Small pipettes are used for transferring the reagents from the bottles, and pouring should never be resorted to, as the lips of bottles are almost invariably dirty.

Reagents which are used in large amounts must be specially freed from iron; Stokes and Cain have therefore limited these to the smallest possible number and to those easily purified. Reagents that are used in very small amounts need not be specially purified, provided the amount of iron present is insufficient to affect the results.

**Ammonia.**—As even the best C. P. ammonia contains notable amounts of iron, it must always be redistilled. The washed ammonia gas is conducted into a cooled ceresine lined bottle containing water, in which it is kept. Only the best white ceresine should be used and care taken to coat the bottle uniformly up to, but not into, the neck.

**Hydrochloric Acid.**—The best C. P. hydrochloric acid invariably contains iron; therefore, always use carefully washed hydrochloric acid gas, prepared by dropping pure concentrated sulfuric acid upon pure ammonium chloride or concentrated hydrochloric acid. Rubber

tubing should be avoided as much as possible, and that which is necessary should be washed out with acid. When practicable, the gas is conducted directly into the solution; when aqueous acid is required it should be freshly prepared.

**Hydrogen Sulfide.**—The use of hydrogen sulfide made directly from iron sulfide is inadmissible and may lead to gross errors. The gas is prepared by dropping acid into a sodium sulfhydrate solution. A stock solution of this is made by saturating 33 per cent sodium hydroxide with hydrogen sulfide and diluting four or five times before using.

**Ammonium Sulfide.**—In general, the sulfhydrate is used and is always freshly prepared by saturating redistilled ammonia with hydrogen sulfide prepared as above.

**Bromine Water.**—This reagent is used to oxidize arsenious and antimonious oxides and to dissolve metals. As iron-free bromine and bromine water, kept in glass vessels, rapidly become contaminated with iron, the bromine water should be prepared as needed by drawing out a clean test tube so as to form a small retort, sucking in 2 or 3 cc. bromine by alternate warming and cooling, and distilling it over into water.

**Nitric Acid.**—When more than 1 or 2 cc. is required, it should be redistilled from a small test-tube retort into a test tube placed in a beaker of water.

**Potassium Permanganate.**—A 1 per cent solution of the best C. P. grade is used. It furnishes the manganese dioxide used as collector for ferric hydroxide, and at the same time serves to oxidize traces of organic matter which might hold it in solution. If not sufficiently free from iron, it may be purified by a manganese concentration.

**Cadmium Sulfate and Chloride.**—These salts are used to supply the cadmium sulfide which serves as collector for iron sulfide, the chloride being used when barium, strontium, or calcium salts are present. A one-fourth molecular stock solution is made, and freed from iron by making a manganese precipitation as described below. The presence of a slight excess of permanganate in the solution has no prejudicial influence.

**Sulfurous Acid, Ammonium Sulfite, Sodium or Ammonium Formate in 1 Per Cent Solution and Alcohol** are used in small quantities to reduce permanganate to manganese dioxide. They need not be specially purified.

**Sodium Potassium Tartrate** is used to hold up alumina or chromic

oxide in concentrating iron from their salts. A 20 per cent stock solution is made and freed from iron by a cadmium sulfide precipitation as described on page 232. The alkaline solution should be neutralized to prevent its action on the glass.

Sodium Hydroxide is used in special cases and its 5 per cent solution must be freed from iron by a manganese concentration. It should be freshly purified unless kept in platinum bottles.

**The Colorimeter.**—The use of the more elaborate and costly colorimeters, with lenses and prisms, is unnecessary, as the accuracy attained by the apparatus described below is quite sufficient, considering the minute amounts dealt with, and the unavoidable errors involved in the methods of concentration and in working with traces of a substance so universally distributed as iron. Moreover, none of the instruments commonly in use enables one to employ an extracting liquid, as is done in the method of Stokes and Cain.

Instead of graduated cylinders of equal diameters, provided with stoppers for the purpose of shaking, ordinary test tubes about 20 cm. long and 24–25 mm. diameter are used. These are carefully selected in pairs, with the aid of calipers. The cross-sections must be as nearly circular as possible and the diameters of the tubes in a pair should not differ by more than 0.1 mm. at corresponding heights, which would give a difference of 0.4 per cent in their readings. Each pair should be carefully numbered and for ordinary purposes one or two pairs are sufficient. The mixing of the liquids is very effectively accomplished by stirrers, one of which is provided for each tube. The stirrer consists of a thin glass rod, bent as shown in Fig. 48 (*a*), into the lower end of which is fused a short platinum wire, attached to a circular disk of platinum, slit radially and bent into the form of a propeller. When not in use the stirrer hangs from the edge of the test tube. It is essential to its proper functioning that the rod when not in use shall hang close to the side of the tube, so as not to interfere with vision; that the platinum disk shall almost, but not quite, touch the bottom, and that it shall work up and down easily. To exclude dust and prevent evaporation, it is well to provide each tube with a heavy, loosely fitting brass cap, perforated to admit the tip of the burette and provided with a radial slit through which the stirrer may pass (Fig. 48 (*b*), *A* and *B*).

In comparing the colors in the two cylinders it is necessary to look through them horizontally, and in order to avoid the effect of the curvature of the glass and of reflection from the inside of the tubes, they

are surrounded by black mantles through which vertical slits are cut on exactly opposite sides. The slits have a height of about  $1\frac{1}{2}$  cm. and a width of  $\frac{1}{2}$  cm. in the side towards the observer and 1 cm. on the opposite side. Notwithstanding the curvature of the glass, slits of this width give a field of practically equal intensity, owing to the refractive action of the liquid. The mantle may be made by rolling thick black paper around the tube, and pasting the edges so that the mantle may

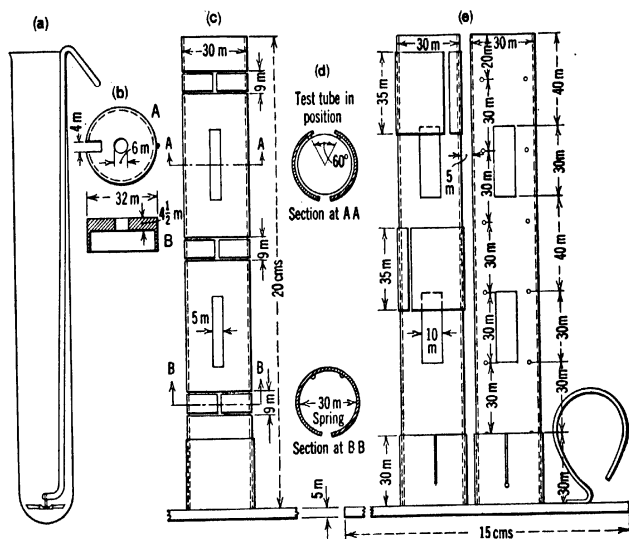


FIG. 48.—Stokes and Cain Colorimeter for Iron.

slip easily over the glass and yet retain its position through friction. Care must be taken that the centers of the slits are exactly opposite, which can be determined by marking the position with the calipers. The two cylinders are mounted in vertical position, as near together as possible, in some form of dark box, such as is used in colorimetric work. Two 10-cc. glass stopcock burettes, carrying the standard iron solution are so mounted that their tips project into the cylinders. In reading, look against a uniformly illuminated vertical sheet of white paper, placed at an angle of about  $45^\circ$  with a window, the degree of the



illumination being regulated by the angle. As there is generally a marked difference in the color sensitiveness of the two eyes, it is necessary, in comparing, to shield or close one eye, the other being opposite the center of the instrument. Stokes and Cain observed that in the great majority of persons, the left eye is more sensitive to red, unless wearied. When much work involving the use of an extracting liquid is to be done, it is convenient to have the mantles made of brass instead of paper and to provide them with springs which will easily hold the cylinders in place, and to mount them side by side in a permanent base.

**Mantles.**—The two mantles are identical in every respect. They are made of 30 mm. brass tubing, of about  $\frac{3}{4}$  mm. thickness, giving an internal diameter of about  $28\frac{1}{2}$  mm. The total height is 20 cm. (Figs. 48(c) and 48(e) and sections Fig. 48(d)).

**Slits.**—Two pairs of slits are provided, for greater convenience in reading with varying volumes of liquid. Their length is 30 mm., the width on one side is 5 mm., on the other 10 mm. The opposite slits must exactly correspond in position and especial care should be taken that their centers are exactly opposite with respect to the axis of the tube; the edges are cut parallel and sharp, not rounded or beveled.

**Collars.**—Each mantle has two thin brass sliding collars, of slit tubing, 35 mm. high, the object of which is to close or vary the height of the slits. They are lined inside with black paper and must slide easily.

**Guide Points.**—Each mantle has 6 pairs of these, at equal distances, as indicated. Their object is to hold the cylinder exactly parallel to the axis of the mantle; it is therefore essential that they shall be exactly in line, parallel to the axis of the mantle, that they shall project into the tube exactly to the same distance, *viz.*, about  $1\frac{1}{2}$  mm., and that they shall have sharp edges or points, not rounded or flat heads. They are made by inserting brass pins or pegs through the wall of the mantle.

**Springs.**—These are intended to hold the cylinder in place against the guide points, and therefore parallel to the axis of the mantle. They are made in pairs, as indicated in Fig. 48, by cutting and bending in a portion of the tube, and should hold a 25 mm. test tube filled with water firmly enough to stay in place, yet so that it can easily be shoved up or down with one hand.

**Mounting.**—Each mantle is supported in a socket, such as is used for

microscope eyepieces, which is mounted on a brass plate 15 cm.  $\times$  10 cm. and not less than 4 mm. thick, provided at one end with a handle for lifting. The mantles must turn easily in the sockets with one hand, and when mounted must be parallel and 5 mm. apart.

Finish.—The whole instrument, except the inside of the sockets, and the portion of the mantles inside them, is coated dull black, within and without.

In using the instrument, the narrow slit is turned towards the observer. In order to prevent reflection from the surface of the glass, the mantles are shielded by surrounding them with a box, made of thick black paper, open at top and bottom, 20 cm. high, 10 cm. deep and  $7\frac{1}{2}$  cm. wide. In the front of this are cut two openings 4 cm. square, at heights corresponding to the slits; in the back at the same height are two pairs of openings 4 cm. high and  $1\frac{1}{2}$  cm. wide, exactly coinciding with the slits in the mantles. When the shield is in place, the rear openings should be invisible from the front. It is also desirable to have the two burettes mounted on a small clamp so that both can be raised or lowered at the same time. A convenient clamp is made by sawing two short pieces of brass tubing across, near the ends, and bending in the pieces so as to form springs. These tubes are soldered to a small vertical brass plate which is attached to a screw muff which supports it on a retort stand. The dimension of the clamp is such that the burette tips coincide with the centers of the cylinders. It is scarcely necessary to add that no iron is permissible, and that the apparatus should be kept free from dust.

**Method and Accuracy of Comparison.**—The cylinders, which may be conveniently designated as the "test" and "standard," after charging in the manner described below contain equal volumes of ether-amyl alcohol mixture and equal volumes of sulfocyanic reagent diluted with equal volumes of water and a few milligrams of potassium persulfate, and are therefore of identical composition and concentration, except that the "test" contains the iron and 2 or 3 mg. manganese and oxidation products of sulfocyanic acid, an amount entirely too small to have any influence on the determination. (Note 3.) They are placed in the colorimeter and the colors are brought to equal intensity by carefully adding to the "standard" a sufficient amount of standard iron solution from the corresponding burette. (Note 4.) The difference of the two burettes is then noted, and the process is repeated three, four, or more times by adding a few drops to one cylinder and

bringing the other to match it. The average of the differences observed is the amount of standard iron equivalent to the iron sought. 1 cc. = 0.005 mg. Fe.

It is well known that extremely faint colors cannot be matched as well as those of somewhat greater intensity. With this method it is possible to determine to within a few per cent an amount of iron so small that it scarcely gives a visible color to the amylic layer. The amylic mixture is used in multiples of 5 cc. and the most favorable conditions appear to be when the volume used is roughly ten times the volume of the standard iron solution equivalent to the iron sought. Under these conditions the extreme differences between the readings should not exceed 5-6 per cent of the total iron present, a result which is much diminished by taking the mean of a series of readings.

Lunge<sup>5</sup> estimates that the permanganate method cannot be depended on to give results nearer than  $\pm 0.14$  mg. Fe. This would mean an error of  $\pm 1$  per cent on 14 mg., or  $\pm 5$  per cent on 2.8 mg. It appears (from the experiments by Stokes and Cain) that the colorimetric method gives an error of less than  $\pm 0.0001$  mg. Fe, or  $\pm 1$  per cent of 0.01 mg., the amount which is conveniently employed in the colorimeter. Since larger quantities of iron can be diluted to any desired extent without introducing an error of this magnitude, it follows that the colorimetric method can be used to advantage up to about 0.014 gram in the absence of interfering substances; above this limit the permanganate method is more accurate. Where a special concentration of the iron is necessary, the error may be estimated on the basis of the results at  $\pm 0.0005$  mg. in the more unfavorable cases, or  $\pm 5$  per cent of 0.01 mg. In such cases the colorimetric method would be applicable, with suitable dilution, up to about 0.0028 gram Fe.

**Concentration Methods.**—The problem of separating a few ten-thousandths of a milligram of iron from several grams of material in a form suitable for determination in the colorimeter is one which must necessarily vary with the nature of the material under examination. In all work with traces of iron it is necessary to exclude dust most carefully, especially where operations which consume considerable time are carried on. All utensils should be carefully rinsed with strong hydrochloric acid just before using; it is well to keep them under hydrochloric acid when not in use, as far as practicable; all funnels and dishes should be kept covered with watch-glasses, which, when removed

<sup>5</sup> Z. angew. Chem., 1896, 3.

should never be placed on the table, but set concave side up, on small glass supports having three supporting glass points; reagent bottles should be kept covered with caps and the contents removed by pipetting rather than by pouring.

Concentration by Evaporation.—When the material is volatile at a sufficiently low temperature and does not attack the vessels the iron may be concentrated by evaporation. This method is applicable to hydrochloric, nitric, sulfuric, and acetic acids, ammonia and other substances of a similar order of volatility. Substances like ammonium sulfate or oxalate and oxalic acid cannot be so treated, as they attack the vessels appreciably. Concentration by evaporation alone is seldom sufficient for bringing the iron into a suitable form for colorimetric determination. Even the purest acids and ammonia are likely to contain traces of colored substances which pass over into the amylic layer and so prevent accurate comparison. In general, after driving off the greatest part of the volatile material, or bringing to dryness and redissolving the residue in a few drops of hydrochloric acid, the iron must be precipitated by one of the methods given below.

The vessels used for evaporation may be of platinum, porcelain, quartz, Pyrex or Jena glass. Except for ammonia and hydrofluoric acid, for which platinum should be used, this metal is unsatisfactory. All platinum contains a small amount of iron, either present as an original impurity or derived from the tools used for working the metal or from materials which have been previously contained in the vessels. This is quite sufficient to cause an appreciable error when acids or ammonium sulfide are evaporated. Berlin porcelain is better than platinum, and Pyrex and Jena glass superior to porcelain. Quartz is excellent, but unnecessary unless extreme accuracy is desired.

Evaporations should be made as rapidly as possible, to diminish the time of action upon the vessel, and entirely out of contact with the external air, so as to avoid dust. It is inadmissible to use any form of apparatus in which the condensed liquid can run or drop back into the vessel. Stokes and Cain found the following form of apparatus entirely satisfactory: A circular disk of asbestos is placed upon a Chaddock's porcelain burner, and upon it is placed an inverted set of porcelain water-bath rings. Upon these rests a 9-inch funnel, the stem of which is drawn out and bent down. Through the stem is forced a current of air which has been filtered by passing through a long tube or series of tubes filled with cotton. A bent calcium chloride tube filled with

cotton is directly connected with the stem of the funnel. The speed of the air current should be such as to prevent condensation in the stem of the funnel. The porcelain rings are slightly inclined so that the condensed liquid running down the sides of the funnel drops off at one point into a beaker. The whole rests on a glass plate. By this means it is possible to evaporate 100–200 cc. concentrated sulfuric acid quietly and rapidly without danger of contamination from dust. Two hundred cubic centimeters distilled water, and 200 cc. redistilled ammonia, evaporated in this apparatus, gave no trace of iron.

The ordinary hemispherical Jena glass evaporating dishes with flat bottoms do not well bear the strain of this treatment. A suitable dish 9½ cm. wide by 4½ cm. high is conveniently made by cutting off the lower part of an 800 cc. Jena Griffen's beaker, and making a lip in it. The dish rests on the porcelain rings, leaving an air space between it and the asbestos. In some cases it is desirable to finish the evaporation on a steam-bath. For this purpose a Berlin porcelain steam-bath is used, with the same funnel. If the steam, as is likely, carries over water containing iron in suspension, it should be passed through a separator, which is conveniently made of a large calcium chloride tube, half filled with beads and provided with an overflow for the water. Combustible liquids like acetic acid cannot be evaporated over the free flame and are evaporated either on the steam-bath or on a small electric hot-plate fitted up as above described.

Concentration by Precipitation.—In by far the greater number of cases it is necessary to concentrate the iron by precipitation. An almost indefinitely small quantity of iron may thus be determined in an indefinitely large amount of material, the only limit being the solubility of the iron precipitate in the solution. It is obviously impossible to collect, on a filter, traces, say a thousandth of a milligram, of ferric hydroxide or sulfide distributed through a considerable volume of an otherwise clear liquid. Stokes and Cain, therefore, employ the method which has been occasionally used successfully in other cases, of mechanically carrying down the precipitate by a relatively large amount of another precipitate, which, when practicable, is generated simultaneously with the iron precipitate. We may designate this secondary precipitate as the "collector." Various substances suggest themselves as collectors; their number is limited by the following considerations. A collector must be sufficiently insoluble, so that but a small amount of a possible impure foreign substance need be introduced;

it must be of such physical consistency as to enable it to carry down all suspended precipitates and must therefore be amorphous and flocculent, not granular or crystalline; it should not be gelatinous or otherwise difficult to wash out in the filter, neither should it be of such consistency as to run through the filter on washing; it must be easily soluble in 7 per cent sulfocyanic acid and must neither interfere with the ferric sulfocyanate reaction nor in the presence of mercuric sulfocyanate impart a color to amyl alcohol, or, if it does not meet these requirements, it must be capable of easy separation from the iron. Aluminum hydroxide would be the ideal collector were it not for the fact that it dissolves slowly and imperfectly in sulfocyanic acid, and thus frequently prevents complete solution of the accompanying ferric hydroxide. Repeated experiments by Stokes and Cain showed that it is not to be depended on, and they have therefore employed it only in special cases where it was removed before final treatment of the precipitate with sulfocyanic acid. The iron is precipitated either as sulfide or as ferric hydroxide. The hydroxide precipitation is employed in the absence of materials which have a solvent action, such as citrates, tartrates, sugar and many other organic substances, pyrophosphates, arsenites, arsenates, antimonates, etc. The usual collector for ferric hydroxide is hydrated manganese peroxide. The sulfide precipitation is used when, from the presence of any of the just-mentioned substances, hydroxide would remain in solution. It is also used when other sulfides insoluble in ammonium or sodium sulfide are practically absent. The best collector for iron sulfide is cadmium sulfide. In this case the cadmium sulfide is redissolved and the iron reprecipitated as hydroxide with manganese dioxide as collector. In many cases the choice between the methods is optional. When there is reason to fear the presence of traces of organic matter, as in the case of materials which have been treated in wooden vessels in the process of manufacture, or when arsenic or other prejudicial substances may be present, as in the cruder reagents, the sulfide method is more accurate. For example, pure sodium chloride gave identical results by either method, while a sample of the best commercial chloride gave decidedly too low results with the hydroxide method.

Special care is necessary in sampling the substance, and wherever practicable, duplicate determinations should be made on portions of the same solution, as it frequently happens that different samples, especially of crystallized substances, taken from the same bottle show

widely varying results, owing to the irregular distribution of the iron.

**Concentration by Manganese Dioxide.**—This is applicable in nearly all cases where substances which have a solvent action on ferric hydroxide, or more than traces of alumina and chromic oxide are absent. The amount and concentration of the substance operated on seem to be immaterial. Stokes and Cain often operated with as much as 50 grams and in solutions as strong as 20 per cent. If the solution is not precipitated by ammonia and contains no substances capable of reducing permanganate to manganese dioxide it is made weakly alkaline with ammonia, about 10 drops of permanganate are added and then 1 to 3 drops of a reducer, such as 1 per cent formate, sulfurous acid or occasionally alcohol, and the solution is then heated a few minutes until the manganese dioxide has separated in flocculent form. It is well to have a slight excess of permanganate. If the substance is one which is precipitated by ammonia, such as zinc, lead, cadmium, just enough of this is added to form a slight permanent precipitate, and the manganese precipitation is made as above. The precipitate is collected on a 5½ or 7 cm. washed filter, and washed a few times with water. Two and a half cubic centimeters of the sulfocyanic reagent are placed in the beaker to dissolve the precipitate adhering to the sides and then dropped carefully around the top of the filter so as to dissolve the manganese dioxide and accompanying ferric hydroxide, the filtrate being run directly into the test cylinder. From 5 to 20 cc. ether-amyl alcohol are added (Note 5) according to the amount of iron present. The beaker is washed out with exactly 10 cc. water, which is poured carefully through the filter. The standard cylinder is charged with 2½ cc. sulfocyanic reagent, 10 cc. water and as much ether-amyl alcohol as was used for the test. Finally, a few milligrams of potassium persulfate are added to each cylinder, and they are transferred to the colorimeter.

For certain special modifications of the above method, see J. Am. Chem. Soc., 29, 427 (1907).

**Concentration by Cadmium Sulfide.**—Cadmium sulfide is used as a collector for iron in the form of sulfide and is applicable in nearly all cases in which the substance under examination either gives little or no precipitate with ammonium sulfide, or one soluble in an excess. Its chief use is to remove the iron as sulfide from solutions which exert a solvent action on ferric hydroxide, and from aluminum and chromium

salts. The following is the method of procedure in the simpler cases:

To the cold solution contained in a Pyrex or Jena beaker, and which should not contain much free acid, are added 2 cc. cadmium solution and then a slight excess of fresh ammonium sulfhydrate, or in case of sulfides soluble in an excess, enough to dissolve these. The liquid is allowed to stand in the cold for about half an hour, with frequent stirring, and the cadmium sulfide, which carries the iron, is then collected on a washed filter and washed a few times with water containing a little ammonium sulfhydrate. The precipitate cannot be directly treated with the sulfocyanic acid reagent, as much iron would be retained and much mercuric sulfide formed; neither can it, as Stokes and Cain have found, be dissolved in bromine water with satisfactory results. It is, therefore, dissolved by carefully dropping hot 1 : 1 hydrochloric acid around the top of the filter, the solution and wash water being run back into the original beaker. The solution, which contains some free hydrogen sulfide is treated as in a manganese concentration, somewhat more than enough permanganate being added to oxidize the hydrogen sulfide first, and then ammonia, the manganous salt in alkaline solution acting as the reducer of the permanganate. In general, the manganese dioxide comes down at once in good form or on gentle heating. The precipitate is collected on the same filter, or if this contains a residue, on a fresh one, and is further treated as described under manganese concentration. In the case of tartrates, oxalates, and other organic substances interfering with the manganese concentration, traces of which remain in the cadmium sulfide precipitate, hot 1 : 1 nitric acid is used in place of hydrochloric acid, and the organic matter is destroyed in the filtrate by adding permanganate to the hot acid solution until the color is permanent, when the solution is made ammoniacal as before.

For special precautions to be observed in particular cases, see J. Am. Chem. Soc., 29, 427 (1907).

**Solubility of Ferric Hydroxide and of Iron Sulfide.**—The accuracy of the above method depends upon the degree of insolubility of ferric hydroxide in the weakly ammoniacal solutions of the various salts. In strong, hot, weakly ammoniacal solutions of certain salts, ferric hydroxide gave the following solubility data:

100 grams $\text{NH}_4\text{Cl}$ dissolved . . . . .	0.0009 mg. Fe or 0.0013 mg. $\text{Fe}_2\text{O}_3$	
100 grams $\text{NH}_4\text{NO}_3$ dissolved . . . . .	0.0009 mg. Fe or 0.0013 mg. $\text{Fe}_2\text{O}_3$	
100 grams $(\text{NH}_4)_2\text{SO}_4$ dissolved . . . . .	none	none
100 grams $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ dissolved . . . . .	none	none



These figures, which are possibly high, if anything, owing to the solvent action of the evaporating acid on the vessel, can lay no claim to accuracy, but they at least show that the loss through solubility will not affect the percentage result of a determination nearer than the seventh decimal place, and may therefore be set off against the slight sources of contamination through dust, solvent action of the reagents on the vessels, etc.

Since determinations made by both of the above methods give practically identical results, it may be concluded that the solubility of iron sulfide, like that of the hydroxide, is negligible. There are certain exceptions to this, especially in the case of stannic salts, but the reader is referred to the original literature.<sup>6</sup>

#### Notes.

1. Even the best grades of ether show traces of peroxide unless especially purified. A sensitive test for peroxide is to shake the ether with freshly reduced acidified ferrous sulfate solution to which has been added sulfocyanate. Ferric sulfocyanate is thus formed and is taken up by the ether.

2. The gradual bleaching of solutions of ferric sulfocyanate has been noted by various observers and is referred to by Tatlock (*loc. cit.*) by Lunge (*loc. cit.*), and by Marriott and Wolf.<sup>7</sup> This is a very usual phenomenon even in ethereal or amylic solutions, and it is not uncommon for two identical tubes, at first matching, to show a very marked difference within less than an hour. This fading, while it may be aided by the action of light, is due to the reduction of the iron to the ferrous state by other substances than normal sulfocyanic acid. Chief among these is isodisulfocyanic acid, which is always formed when sulfocyanates are acidified, and which reduces ferric salts with great rapidity. Hydrocyanic acid and hydrogen sulfide, both of which are decomposition products of sulfocyanic acid, may possibly also take part. Hydrocyanic acid reduces traces of ferric salts, which are reoxidized by persulfate. A weak solution of ferric sulfocyanate in water or amyl alcohol is decolorized on boiling for a few moments and hydrogen sulfide can be detected in the escaping vapors.

The addition of a few milligrams of persulfate keeps the iron in the

<sup>6</sup> J. Am. Chem. Soc., **29**, 427 (1907).

<sup>7</sup> J. Biol. Chem., **1**, 451 (1906).

oxidized state, even in the presence of small amounts of hydrogen sulfide or sulfurous acid. Its use, however, has one striking disadvantage. Like hydrogen peroxide, but less rapidly, it oxidizes sulfocyanic acid, forming a yellow substance which is taken up by the amyl alcohol, often rendering even an approximate comparison impossible. It appears that this yellow body does not proceed from normal sulfocyanic acid itself, but from other substances, possibly the still unknown isosulfocyanic acid, which may accompany it in small quantity. Sulfocyanic acid freshly prepared by decomposition of its silver or mercury salt by hydrogen sulfide gives but little of the yellow body with persulfate. If, on the contrary, its 5 to 10 per cent solution has been allowed to stand for some time, or if a freshly acidified solution of a sulfocyanate be treated with persulfate, the amylic extract is always colored. Be the cause what it may, there is always enough of the yellow substance formed by persulfate to render an accurate comparison impossible. Even without persulfate a sulfocyanic acid which has stood for a few days always contains enough yellow substance to make it useless.

The addition to the sulfocyanic acid of a sufficient amount of mercuric sulfocyanate to form the double compound,  $\text{Hg}(\text{SCN})_2 \cdot 2\text{HSCN}$ , not only totally inhibits the action of persulfate, but also preserves the acid indefinitely against injurious changes, while it does not appreciably diminish the sensitiveness of the reaction with ferric salts. The amylic solution has a perfectly pure color from the start and in the presence of a trace of persulfate and occasional stirring retains its intensity of color absolutely unchanged for many hours. The addition of persulfate is necessary, as mercuric sulfocyanate does not prevent the fading of the ferric sulfocyanate. The introduction of mercury causes certain complications. These are treated in their proper places under the separation of iron from the various metals: See J. Am. Chem. Soc. **29**, 427 (1907).

3. To one of two carefully matched cylinders was added the manganese dioxide from 0.6 cc. 1 per cent permanganate, about the quantity used in a concentration. Not the least change could be detected. After adding 40 mg. sulfuric acid to one of the matched cylinders, 0.14 cc. standard iron solution had to be added to the same cylinder to restore equality. It therefore appears that as little as 40 mg. sulfuric acid may produce an error of 0.0007 mg. Fe. Since the sulfuric acid generated by the above amount of manganese does not exceed 1.2 mg.,

its influence is clearly too small to be detected, amounting to only perhaps 0.004 cc. standard solution.

4. When the amount of standard added equals 1 cc., it is well to add an approximately equal volume of water to the "test" cylinder, so as to keep the volumes equal and counteract the unequal solvent action on the ether-amyl alcohol layer.

If the concentration of the iron has been properly performed the quality of the colors will be identical. A yellowish cast in the "test" is due to faulty concentration, and an accurate comparison cannot then be made.

As we read by looking through the amylic layer it is essential that this shall be perfectly clear and free from suspended water drops; the turbidity of the aqueous layer through suspended amyl alcohol is of no significance. If the stirring is properly performed the amylic layer becomes rapidly clear and the aqueous layer remains turbid. Whether or not this will be realized can be instantly told by observing the manner in which the separation occurs. If the churning be thorough, large globules will be seen on the upper surface, which will be seen to coalesce rapidly, after the manner of bubbles, leaving a perfectly clear amylic layer, while below, the mixture contains innumerable small drops which do not run together but gradually rise, leaving a turbid aqueous layer. If, however, the churning has been imperfect the large globules are at the bottom and run together rapidly, leaving a sharply defined surface and a clear aqueous solution, while above are seen small globules which gradually fall, leaving the amylic layer turbid. In general, the former effect takes place; if it does not, even with sufficient churning, it can be brought about by adding more water to each cylinder.

5. The ether-amyl alcohol should be added before adding water, as otherwise there is likely to be a separation of mercuric sulfocyanate. The amount to be added can be judged by the color; it is better to add too little than too much, as more can be added later, if desired. If the amount of iron is very considerable, so as to require more than 5 cc. standard iron solution, the filtrate can be diluted in a measuring flask and an aliquot portion taken, a fresh portion of sulfocyanic reagent being used.

## DETERMINATION OF IRON AS THE SULFOCYANATE

## METHOD OF MELLOR

Like the preceding method of Stokes and Cain, this method makes use of an ether-amyl alcohol solution for extracting ferric sulfocyanate. It is recommended by J. W. Mellor<sup>8</sup> for the determination of iron in china clay. "With some of the china clays exceptional manipulation is required. Twice as much  $\text{Fe}_2\text{O}_3$  may appear in the analysis as is actually present in the clay."

**Reagents.**

1. Potassium sulfocyanate. Dissolve 97 grams of the pure salt (recrystallized) in water and make up to a liter.
2. Potassium aluminum sulfate. Fuse 0.05 gram  $\text{Al}_2\text{O}_3$  in 5 grams of  $\text{KHSO}_4$ , dissolve in water and dilute to a liter.
3. Ether-amyl alcohol solution. Mix 5 volumes of methylated ether with 5 volumes of iso-amyl alcohol.
4. Standard iron solution. Dissolve 0.0315 gram of ferric potassium alum,  $\text{Fe}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ , in water, add 5 cc. of concentrated sulfuric acid, cool, and dilute to a liter. Mix thoroughly. One cubic centimeter contains 0.005 mg. of  $\text{Fe}_2\text{O}_3$ .

**Procedure.**—The clay is fused with  $\text{KHSO}_4$ , the melt taken up in water, made up to a suitable concentration, and an aliquot part of 5 cc. put in one of two color comparison tubes. Add 5 cc. of potassium sulfocyanate solution and 10 cc. of ether-amyl alcohol solution. To the other tube add 5 cc. of potassium aluminum sulfate solution, 5 cc. of potassium sulfocyanate solution, and 10 cc. of ether-amyl alcohol. Then run the standard iron solution from a burette into the standard tube, stirring gently after the addition of each 0.1 cc. Dilute the test solution every 1 cc. to the same volume as that of the standard. Make the comparison in a color camera.

**Notes.**

1. The ferric sulfocyanate is taken up in the ether-amyl alcohol layer while interfering salts are retained by the water layer.
2. No claim is made to such a high degree of accuracy as is attainable with the method of Stokes and Cain. Mellor, however, recommends it as a satisfactory method for estimating iron in china clay.

<sup>8</sup> Trans. Ceram. Soc. England, 8, 125 (1908-9).

**DETERMINATION OF IRON AS FERRIC CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID**

This determination is based upon the fact that ferric chloride gives an intense yellow solution when dissolved in concentrated hydrochloric acid. The color is of maximum intensity when the hydrochloric acid is about 28 per cent.

**Reagents.**

1. Hydrochloric acid. Thoroughly washed hydrogen chloride gas is collected directly in a cooled bottle containing distilled water and the solution diluted to 28 per cent HCl content. The gas is obtained by boiling concentrated hydrochloric acid or by dropping pure concentrated sulfuric acid upon pure sodium or ammonium chloride. Rubber connections should be avoided, or at least thoroughly washed with acid before use. Water saturated with HCl at room temperature contains about 40 per cent of acid by weight. The specific gravity of the solution is 1.20.

2. Standard iron solution. Iron wire for standardizing volumetric solutions may be used. This wire usually contains from 99.5 to 99.8 per cent of iron. Weigh an amount to contain exactly 1 gram of iron, dissolve it in nitric acid, add hydrochloric acid, and evaporate to dryness on a water-bath. Take up the residue in a little hydrochloric acid and again evaporate to dryness. Once more add hydrochloric acid and evaporate to dryness. By this repeated treatment with hydrochloric acid, all of the nitric acid is driven off. To the final residue add a 28 per cent solution of hydrochloric acid to give exactly 100 cc. of solution and thoroughly mix. Ten cubic centimeters of this solution are diluted to a liter with 28 per cent hydrochloric acid and thoroughly mixed. One cubic centimeter of the resulting solution contains 0.0001 gram of iron.

**Procedure.**—Dissolve in a 28 per cent solution of hydrochloric acid a weight of the sample such that the final volume contains not more than 0.0001 gram of iron per cubic centimeter and preferably less than half this amount. The solution is then matched in color against the standard iron solution by the method of balancing. If the sample is insoluble in hydrochloric acid, dissolve in aqua regia or nitric acid, and remove all nitrates by repeated evaporation to dryness with concentrated hydrochloric acid, finally taking up the residue in a con-

venient volume of 28 per cent hydrochloric acid and balancing the resulting color against the standard solution.

#### Notes.

1. If a large number of analyses are to be made, the method of balancing should be used so as to economize with the concentrated hydrochloric acid required for diluting.

2. Free chlorine does not interfere with the analysis, but oxides of nitrogen do. Hence, every trace of nitrate and oxides of nitrogen must be removed when nitric acid is used to dissolve the sample. This is only accomplished by repeated evaporation of the solution with concentrated hydrochloric acid. Anhydrous cupric chloride in concentrated hydrochloric acid gives a yellow-colored solution similar to that of ferric chloride. Hence, copper must be removed by precipitation with hydrogen sulfide. In concentrations less than 1 in 20,000, cobalt and nickel do not interfere. Manganese has no influence on the test. Even large amounts of this element give colorless solutions in concentrated hydrochloric acid.<sup>9</sup>

#### DETERMINATION OF IRON AS SULFIDE

When ammonium sulfide, or hydrogen sulfide and ammonium hydroxide, is added to a solution containing ferrous iron, the solution turns brown due to the formation of ferrous sulfide. The method may be used to estimate ferrous iron in the presence of ferric iron or to estimate the total iron. When the total iron content is desired, the ferric iron is reduced to the ferrous condition before adding the ammonium sulfide, or hydrogen sulfide and ammonia.

#### Reagents.

1. Hydrochloric acid. Thoroughly washed hydrogen chloride gas is collected directly in a cooled bottle containing distilled water. The gas is obtained by boiling concentrated hydrochloric acid or by dropping pure concentrated sulfuric acid upon pure sodium or ammonium chloride. Rubber connections should be avoided, or at least thoroughly washed with acid before use.

2. Ammonium hydroxide. It is necessary to redistill the ammonia unless a "blank" test shows iron absent. The best C. P. ammonium

<sup>9</sup> C. Hüttner, *Z. anorg. Chem.*, **86**, 341 (1914).

hydroxide is distilled and the washed ammonia gas collected directly in a ceresine lined bottle containing distilled water, the bottle being kept cool in ice cold water or cold running tap-water. Care must be taken to thoroughly line the bottle up to, but not into, the neck, and only the best grade of white ceresine should be used.

3. Hydrogen sulfide. The gas is prepared by dropping acid into a solution of sodium sulfhydrate. The gas is washed by bubbling through a bottle containing water. The sodium sulfhydrate is made by saturating a 33 per cent sodium hydroxide solution with hydrogen sulfide. This solution is diluted four or five times before using. Hydrogen sulfide obtained directly from iron sulfide should not be used on account of the danger of introducing iron into the test solution.

4. Ammonium sulfide. The solution is freshly prepared as needed by saturating redistilled ammonia with hydrogen sulfide prepared as described above.

5. Standard iron solution. Dissolve 0.7022 gram of ferrous ammonium sulfate,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , in freshly boiled distilled water, containing 2 cc. of concentrated sulfuric acid, and saturated with pure hydrogen sulfide. Dilute to a liter, using water saturated with hydrogen sulfide, and thoroughly mix. The bottle must be tightly stoppered and must be recharged with hydrogen sulfide when the odor becomes faint. One cubic centimeter of this solution contains 0.0001 gram of ferrous iron.

**Procedure for Ferrous Iron.**—A sample of the test substance is taken such that it contains between 0.0015 and 0.0003 gram of iron per liter. One hundred cubic centimeters of the sample and 95 cc. of distilled water are each placed in a Nessler tube. Five cubic centimeters of hydrogen sulfide water and 2 drops of ammonium hydroxide are added to each tube and the contents thoroughly mixed. The standard iron solution is then added drop by drop to the "blank" until the color approximately matches that of the test solution. The color of the test solution is brown, while the standard has a bluish-black tinge.<sup>10</sup> A few drops of hydrochloric acid are added to discharge the color of the standard and then ammonia added drop by drop until the color reappears. The color is now a brown of the same tinge as that of the test solution. If necessary, add more standard iron solution until the two solutions are matched. Should the standard now show a slightly different tinge from that of the test solution,

<sup>10</sup> L. W. Winkler, *Z. anal. Chem.*, **41**, 550 (1902).

decolorize with hydrochloric acid and retreat with ammonia as before. The color of both the standard and the sample should now be identical.

**Procedure for Total Iron.**—If the sample is a liquid, add a few cubic centimeters of hydrochloric acid, a pinch of potassium chlorate and evaporate to dryness on a water-bath; if a solid, dissolve in water or acid, add hydrochloric acid and potassium chlorate, and evaporate on a water-bath. Dissolve the residue in a little warm water acidified with 1 or 2 cc. of hydrochloric acid and add 5 cc. of water saturated with hydrogen sulfide. Filter the solution to free it of particles of sulfur and dilute to 100 cc., 500 cc., or 1000 cc. Thoroughly mix and proceed with an aliquot part as directed above under "Procedure for Ferrous Iron," adding the ammonia drop by drop until the solution is just neutral to litmus paper and then two drops more.

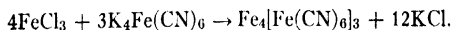
#### Notes.

1. The quantity of sample taken for the final matching should be such as to contain between 0.00015 gram and 0.0003 gram of iron.

2. The following metals, whose sulfides are colored, interfere with the analysis: Lead, silver, mercury, copper, bismuth, cadmium, arsenic, antimony, tin, cobalt, and nickel. The first nine of these metals may be removed in the usual way by precipitating in 0.3 N acid solution with hydrogen sulfide. The hydrogen sulfide should be prepared as described above under "Reagents." The iron may be separated from cobalt and nickel by precipitation as ferric hydroxide, filtering, and dissolving the hydroxide in a little dilute hydrochloric acid. It must be remembered that in the use of hydrogen sulfide the iron, if present in the ferric condition, is reduced; and before the precipitation with ammonia the iron must be oxidized to the ferric state. The iron may be oxidized, after removal of hydrogen sulfide if present, by boiling several minutes with a few cubic centimeters of nitric acid or bromine water.

#### DETERMINATION OF IRON BY POTASSIUM FERROCYANIDE

This determination is based upon the intense blue ferric ferrocyanide (Prussian blue) formed when a solution of potassium ferrocyanide is added to solutions of *ferric* salts.





It may be used to estimate ferric iron in the presence of ferrous iron, or to estimate the total iron. The reaction is an extremely delicate one and, hence, can be employed only for very low concentrations of iron, such as are found in water, or "traces" of iron in salts.

### Reagents.

1. Potassium ferrocyanide. Dissolve 5.0 grams of pure potassium ferrocyanide in a liter of water.

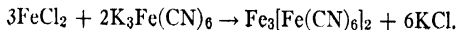
2. Standard iron solution. Dissolve 0.1404 gram of ferrous ammonium sulfate,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , in water containing 5 cc. of concentrated sulfuric acid, oxidize the iron with potassium permanganate, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 0.02 mg. of iron. If the results are to be reported as  $\text{Fe}_2\text{O}_3$ , then use 0.1474 gram of salt per liter. This solution contains 0.03 mg. of  $\text{Fe}_2\text{O}_3$  per cubic centimeter.

**Procedure for Ferric Iron.**—Adjust the solution to be tested, by evaporating or diluting, so that its iron content is approximately 0.002 gram per liter. Measure out a 50 cc. portion, add 5 cc. of the potassium ferrocyanide solution, thoroughly mix, and compare the color with the standard, preferably by the method of balancing or that of dilution. The standard is prepared by adding 10 cc. of the potassium ferrocyanide solution to 10 cc. of the standard iron solution, diluting to 100 cc. and mixing. One cubic centimeter of this standard contains 0.002 mg. of Fe or 0.003 mg. of  $\text{Fe}_2\text{O}_3$ , depending upon which weight of salt was taken in making the standard iron solution.

**Procedure for Total Iron.**—The procedure for determining the total iron content is the same as that for the ferric iron, except the solution of sample is acidified with sulfuric acid and treated with permanganate to oxidize the ferrous iron. The potassium ferrocyanide solution is then added and the color matched against the standard as directed in the previous procedure.

### DETERMINATION OF FERROUS IRON BY POTASSIUM FERRICYANIDE

This method is based upon the formation of Turnbull's blue (indistinguishable in color from Prussian blue) when solutions of *ferrous* salts and ferricyanide are mixed.



**Reagents.**

1. Sulfuric acid, 6 N. Pour 1 volume of concentrated sulfuric acid, sp. gr. 1.84, into 5 volumes of distilled water and stir. Be sure the acid is iron-free.

2. Potassium ferricyanide. Dissolve 0.5 gram of pure potassium ferricyanide crystals and dilute to a volume of 100 cc. The solution must be freshly prepared.

3. Standard ferrous iron solution. Dissolve 0.7022 gram of crystallized ferrous ammonium sulfate,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , in a large volume of freshly boiled distilled water to which 10 cc. of 6 N  $\text{H}_2\text{SO}_4$  has been added, dilute to a liter and mix. This solution contains 0.1 mg. of iron per cubic centimeter. It must be freshly prepared.

**Procedure.**—"Add 10 cc. of dilute sulfuric acid to 50 cc. of the sample, remove the suspended matter by filtration if necessary, and add 15 cc. of potassium ferricyanide solution. Dilute to the mark in a 100 cc. Nessler tube with distilled water that has been freshly boiled and cooled. Compare the color developed in the sample with that in standards made at the same time from the ferrous iron solution, in this way: Place in 100 cc. Nessler tubes, in the following order, 75 cc. of distilled water, 10 cc. of dilute sulfuric acid, and 15 cc. of ferricyanide solution, and mix well the contents of each tube. Add various volumes of standard ferrous iron solution to several tubes, mix well, and compare immediately the resulting colors with that of the sample."<sup>11</sup>

**Note.**—Comparison of color developed in both sample and standards must be made in matched Nessler tubes in the presence of equivalent concentrations of acids, immediately after the reagent is mixed with the solutions. The color is deepened by an excess of the reagent, is diminished by an excess of acid, and fades quickly on standing.

**DETERMINATION OF IRON BY SALICYLIC ACID**

Solutions of ferric iron are colored amethyst by the addition of

salicylic acid,  $\text{C}_6\text{H}_4 \begin{cases} \text{OH} & (1) \\ \text{COOH} & (2) \end{cases}$  while solutions of ferrous iron remain

colorless. This reagent, therefore, offers a means of estimating ferric iron in the presence of ferrous iron. By oxidizing the ferrous iron

<sup>11</sup> Standard Methods of Water Analysis, 6th ed., p. 49. American Public Health Association, New York, 1925.

present, the method may also be used to determine the total iron content.

#### Reagents.

1. Salicylic acid. Use a saturated solution.
2. Standard iron solution. Dissolve 0.0864 gram of ferric ammonium alum,  $\text{Fe}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ , in distilled water containing 2 cc. of concentrated sulfuric acid, dilute to a liter and thoroughly mix. One cubic centimeter of this solution contains 0.00001 gram of  $\text{Fe} \approx 0.000014$  gram of  $\text{Fe}_2\text{O}_3$ .

**Procedure for Ferric Iron.**—If the substance is a solid, dissolve in 20 cc. of water an amount sufficient to have an iron content between 0.00001 and 0.0002 gram; if a liquid, adjust the concentration by dilution or evaporation to give a solution containing between 0.00001 gram and 0.0002 gram of iron per 20 cc. Filter in case the solution is turbid, provided it is known that no iron is present in the suspended particles. In case the sample is insoluble in water, or only partly soluble, dissolve in hydrochloric acid, using as small an excess as possible and then neutralize the excess with ammonium hydroxide. The total volume of the solution, after neutralization, should not be over 20 cc. Now add 5 cc. of salicylic acid solution to the sample and compare at once the color with that of a standard iron solution, to which has just been added 5 cc. of the salicylic acid solution. Comparison should be made either by the method of balancing or the method of dilution.

**Procedure for Total Iron.**—Dissolve the sample as directed in the "Procedure for Ferric Iron," slightly acidify the solution with sulfuric acid, and oxidize the ferrous iron with potassium permanganate. Then neutralize the free acid with ammonium hydroxide, add 5 cc. of salicylic acid solution and match the color at once with that of a standard to which has just been added 5 cc. of salicylic acid solution.

#### Notes.

1. If more than 0.0002 gram of iron is present in the sample, the color produced by the salicylic acid is too intense for colorimetric work. With less than 0.00001 gram, the color is too pale for accurate comparison.
2. The method can be used to estimate small quantities of iron in salts. The following substances interfere with the accuracy of the

analysis: phosphates, thiosulfates, sulfites, bisulfites, fluorides, and free mineral acids. When it has been necessary to use acid to dissolve the sample, or when the solution of sample is acidified and oxidized with permanganate, the excess acid must be neutralized with ammonia before adding the salicylic acid.

3. If the sample contains organic matter, heat to dryness with nitric acid, ignite, and dissolve the residue as directed for the solution of sample when organic matter is absent. Remember that after heating with nitric acid, and igniting, the iron is in the ferric state. To insure complete oxidation in the final solution, however, it is advisable to treat the slightly acidified solution with a little permanganate, and then neutralize with ammonia.

4. In neutralizing with ammonia, care must be taken not to add an excess. Should an excess accidentally be added, precipitating the iron as hydroxide, make the solution slightly acid and then add, drop by drop, dilute ammonium hydroxide until the neutral point is just reached.

5. The color produced by the salicylic acid and ferric iron fades fairly rapidly in the light. Hence the necessity of adding the salicylic acid to the sample and standard at the same time and comparing at once. The method of duplication is not to be recommended on account of the time required, during which fading may occur. For the same reason a comparison with a series of standards is not made.

#### DETERMINATION OF IRON BY ACETYLACETONE

Acetylacetone,  $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{CH}_3$ , gives an intense red coloration with ferric salts, due to the formation of ferric acetylacetone, one of the hydrogens attached to the middle carbon being replaced by the metallic ion.<sup>12</sup> In very dilute solutions the color is orange-red by transmitted light and yellow by reflected light. Stronger solutions give a deep red color by transmitted light and orange-red by reflected light.

##### Reagents.

1. Acetylacetone. A 0.5 per cent solution is made by diluting with water or weak alcohol freshly distilled acetylacetone. Two cubic centimeters of this solution will give an excess of acetylacetone for as large an amount of iron as can be determined by this method.

<sup>12</sup> Combes, *Compt. rend.*, 105, 868 (1887).

Acetylacetone is a colorless liquid, having a boiling-point of 137° C.

2. Standard iron solution. Dissolve 0.1404 gram of ferrous ammonium sulfate,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , oxidize the iron with potassium permanganate, and dilute to a liter. One cubic centimeter of the solution contains 0.00002 gram of iron. Standard iron solutions of various strengths may also be made by dissolving the purest iron wire in a few cubic centimeters of nitric and hydrochloric acids and diluting to the desired volume.

**Procedure.**—The sample is treated on the water-bath with a few drops of sulfuric and nitric acids to remove any organic matter and to oxidize the iron. The amount of sample taken should be such as to contain between 0.00005 gram and 0.0006 gram of iron. If the whole of the sample is to be used, the acid must be removed by evaporation so that when it is diluted to 50 cc. in the Nessler tube the solution is only *faintly* acid. (*Not* acid enough to give the tube a pink tinge when viewed from the side. See Note 2.) If the iron content is high, so that it is necessary to dilute to 500 cc. or to 1000 cc. and to take an aliquot part, then it is not necessary to remove the small amount of acid. The sample, or an aliquot part of it, is transferred to a Nessler tube, 2 cc. of 0.5 per cent acetylacetone solution added, the tube filled to the mark with distilled water, and the contents thoroughly mixed by pouring back and forth into a clean beaker. This solution is then matched with a standard made by putting 2 cc. of 0.5 per cent acetylacetone into a Nessler tube, partly filling with distilled water and adding sufficient standard iron solution to match the sample after diluting to the mark with water and mixing.

#### Notes.

1. The smallest amount of iron that can be detected with acetylacetone is 0.000003 gram. The largest amount that can well be estimated in a 50 cc. tube is about 0.0006 gram. The range of greatest accuracy is from 0.00005 gram to 0.0006 gram. With a tube containing 0.00005 gram of iron, it is not difficult to detect a variation of 0.0000025 gram, and for amounts of about 0.0004 gram a variation of 0.00001 gram is easily detected.

2. Very few of the common inorganic salts have any influence on the color when present in small amounts. Two-tenths of a gram of substances, which will furnish the following ions, have no effect on the

color: Na, K, Ba, Sr, Ca, Mg, Mn, Zn, Al, Hg, As, Cd, Pb, Cl, Br,  $\text{SO}_4$ ,  $\text{NO}_3$ , and  $\text{ClO}_3$ . Smaller amounts of copper, phosphoric acid, and silicic acid do not interfere. Dissolved carbon dioxide has no influence. Ammonia does not precipitate the ion, but the solution is colored yellow. This yellow coloration, however, does not prevent estimating the iron by its characteristic color. Oxides of nitrogen must be removed by boiling the concentrated solution, since they give a brown color with acetylacetone. Sodium and potassium hydroxides destroy the ferric acetylacetone, the iron precipitating as ferric hydroxide. Not more than one drop of any of the common dilute acids should be present at the final dilution in the Nessler tube. One drop of a strong acid added to the faintly acid solution is sufficient to weaken and change the color of the ferric acetylacetone. In no case must the solution when diluted to the mark in the Nessler tube be acid enough to give a pink tinge when viewed from the side.

3. The color produced by ferric ions and acetylacetone in faintly acid solution is quite permanent. Solutions so dilute as to show only brownish or orange-red tinge by transmitted light and a yellowish tinge by reflected light showed no change in three weeks when compared with freshly made solutions. The color is slightly altered by strong sunlight. Small temperature changes have no appreciable effect on the color. Boiling will change the shade of color, but upon cooling the solution the original color reappears.

4. The method of balancing cannot be used (without a calibration curve), since the color of ferric acetylacetone solutions does not vary uniformly with the height of the column of the liquid. For example, pouring out half of the solution from a filled tube will not leave a color equal to the color in a filled tube but containing the same amount of iron as is in the half filled tube.

5. It is important for accurate work that the amount of free acid, the excess of acetylacetone, and the volume of the final solutions be the same in both the test solution and the standards.

6. A series of standards of various strengths may be made from the standard iron solution and kept for continuous use. It is, of course, necessary to protect them from dust and direct sunlight.

7. Conductivity measurements made by Hantzsch and Desch<sup>13</sup> indicate that in water solution ferric acetylacetone is only slightly dissociated. "It is, however, largely hydrolyzed into ferric hydroxide

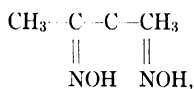
<sup>13</sup> Ann., **323**, 1 (1902).

and acetylacetone,"<sup>14</sup> hence the necessity of having the concentrations of acid and acetylacetone, and the volume, the same in all color comparisons. The purpose of the acid is, of course, to repress the hydrolysis.

8. Urbain and Debiere<sup>15</sup> state that ferric acetylacetone is almost insoluble in water. Pulsifer<sup>16</sup> found a solubility of 1.5 grams per liter of solution.

#### DETERMINATION OF IRON BY DIMETHYLGLYOXIME

When an alcoholic solution of dimethylglyoxime,



is added to a solution containing ferrous iron, a bright red color is produced due to the formation of  $\text{Fe}(\text{C}_4\text{H}_7\text{N}_2\text{O}_2)_2$ , one of the hydrogens attached to oxygen being replaced by an equivalent of iron. The iron is conveniently reduced by means of a little hydrazine sulfate,  $\text{N}_2\text{H}_4\cdot\text{H}_2\text{SO}_4$ .

#### Reagents.

1. Dimethylglyoxime. Saturate 95 per cent ethyl alcohol with the purest dimethylglyoxime.

2. Hydrazine sulfate. Use the solid salt purified by recrystallization from its water solution.

3. Ammonium hydroxide. A 25 per cent solution of ammonia, obtained by diluting redistilled ammonia prepared as outlined on page 239.

4. Standard iron solution. Dissolve 0.1404 gram of ferrous ammonium sulfate,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4\cdot 6\text{H}_2\text{O}$ , in distilled water containing 5 cc. of concentrated sulfuric acid, oxidize with potassium permanganate solution, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 0.00002 gram of iron. If it is desired to report the results in terms of  $\text{Fe}_2\text{O}_3$ , then use 0.1474 gram of salt instead of 0.1404 gram. This will give a liter of solution containing 0.00003 gram of  $\text{Fe}_2\text{O}_3$  per cubic centimeter. If the standard is

<sup>14</sup> H. B. Pulsifer, J. Am. Chem. Soc., **26**, 967 (1904).

<sup>15</sup> Compt. rend., **120**, 302 (1899).

<sup>16</sup> *Loc. cit.*

to be used for the method of balancing or that of dilution, it will be necessary to dilute to one-tenth of its concentration. This is conveniently done by diluting 25 cc. of the standard (containing 0.1404 gram of salt per liter) to 250 cc. One cubic centimeter of this solution contains 0.000002 gram Fe. If the standard contained 0.1474 gram of salt per liter, then 25 cc. diluted to 250 cc. will contain 0.000003 gram of  $\text{Fe}_2\text{O}_3$  per cubic centimeter.

**Procedure.**—The sample should have an iron content between 0.01 and 0.06 gram per liter. Take 50 cc. of the sample, add 1 gram of hydrazine sulfate, 5 cc. of the dimethylglyoxime solution, and heat to boiling. Then add 10 cc. of 25 per cent ammonia solution, continue boiling a half minute, cool rapidly, and dilute to 100 cc. for comparison. The color matching may be made by any of the four usual methods.

#### Notes.

1. The iron may be estimated with an accuracy of 0.5 per cent of the amount present in the solution. By concentrating or diluting, the iron content should be adjusted to between 0.01 and 0.06 milligram per cubic centimeter. The minimum amount of iron that can be detected is less than 0.00005 mg.

2. The alkali and alkaline-earth metals do not interfere with the analysis. Magnesium may introduce a small error, if present in *relatively* large amount. Tschugaeff and Orelkin<sup>17</sup> give the following experimental results:

- I. Solution contains 0.000848 gram Fe + 0.01995 gram Mg (as sulfate) per 100 cc.
- II. Solution contains 0.000606 gram Fe + 0.03325 gram Mg (as sulfate) per 100 cc.
- III. Solution contains 0.000303 gram Fe + 0.04987 gram Mg (as sulfate) per 100 cc.

Fe PER 100 Cc.

Present, Gram	Found, Gram	Per Cent Difference
I. 0.000848	0.000854	0.7
II. 0.000606	0.000606	0.0
III. 0.000303	0.000309	2.0

The analysis cannot be made in the presence of *relatively* large amounts of aluminum or zinc.

<sup>17</sup> Z. anorg. Chem., **80**, 401 (1914).



## CHAPTER XXI

### LEAD

#### DETERMINATION OF LEAD AS THE SULFIDE

WHEN hydrogen sulfide, ammonium sulfide, or alkali sulfides are added to very dilute solutions of lead salts, the solutions are colored brown because of the presence of lead sulfide in colloidal suspension. Other metals whose sulfides are colored must be absent. Since colloidal suspensions are very sensitive to electrolytes, the concentration of the latter must be kept as small as possible and the same in both standard and sample. The coagulating action of the electrolytes may be prevented (or very much reduced) by forming the lead sulfide in the presence of gelatin or sugar.

#### METHOD A.—FORMATION OF PbS IN ACID SOLUTION

##### Reagents.

1. Nitric acid, 6 N.
2. Sodium acetate, 3 N solution.
3. Sugar solution. Use a 50 per cent solution made from pure cane sugar.
4. Gelatin solution. Use a 1 per cent solution of pure gelatin.
5. Hydrogen sulfide. Make a fresh solution by saturating water with thoroughly washed hydrogen sulfide. (See page 240.)
6. Standard lead solution. Dissolve 0.1599 gram of pure lead nitrate,  $\text{Pb}(\text{NO}_3)_2$ , in water, add a few drops of nitric acid, dilute to 1 liter and mix thoroughly. This solution contains 0.1 mg. of lead per cubic centimeter.

**Procedure.**—If the sample is a solid, weigh out an amount that contains between 0.005 and 0.25 mg. of lead, dissolve in 20 cc. of nitric acid, boil off the excess of acid, and dilute to 35 cc. If the sample is water, a measured sample containing between 0.005 and 0.25 mg. of lead is evaporated to a volume of about 35 cc. To the 35 cc. of solution add 3 drops of sodium acetate solution, 2 cc. of 6 N nitric acid,

10 cc. of 50 per cent sugar solution (or 1 cc. of the gelatin solution), dilute to 50 cc., and mix thoroughly. Then add 2 cc. of freshly prepared hydrogen sulfide solution and mix *gently* by means of a glass plunger. Compare the color at once with that of a standard lead sulfide suspension prepared along with the sample and under identical conditions as to concentration of reagents, order of mixing, etc. The comparison of color may also be made by the dilution and balancing methods. The standard lead sulfide solution is prepared by diluting 10 cc. of the standard lead solution with about 700 cc. of water, and adding 200 cc. of 10 per cent sugar solution (or 20 cc. of 1 per cent gelatin solution), 3 cc. of sodium acetate solution, 40 cc. of 6 N nitric acid, 10 cc. of hydrogen sulfide, and water to make 1 liter. Mix thoroughly but gently. One cubic centimeter of this solution contains 0.001 mg. of lead.

If a number of determinations are to be made, a set of permanent standards can be prepared by mixing in the proper proportions solutions of copper, cobalt, and ferric sulfates (or chlorides). These standards are matched against freshly prepared lead sulfide solutions and then sealed in glass cylinders. For the preparation of permanent standards from colored inorganic salts, see H. V. Arny and C. H. Ring, *J. Ind. Eng. Chem.*, **8**, 309 (1916).

#### Notes.

1. Since the lead sulfide is in colloidal suspension, the intensity and shade of the brown color will depend upon the number and size of the lead sulfide particles. Hence, it is necessary that the sample and standard sulfide suspensions be prepared under as nearly the same conditions as possible. The mixing should be carried out gently so as not to cause agglomeration of the lead sulfide.

2. The color of the sulfide suspensions will remain unchanged for at least half an hour if exposed to full daylight only when being compared.

3. A few drops of nitric acid are added to the standard lead nitrate solution to prevent formation and precipitation of a basic salt.

4. The analysis is hindered by the presence of a considerable amount of iron. The latter cannot be removed by precipitation with ammonium hydroxide, since the ferric hydroxide will carry down a part or all of the lead. In case iron interferes, use the alkaline sulfide method on page 253.

5. The sodium acetate solution is added before adding the hydrogen sulfide so as to obtain a clear brown solution and not a turbid one. Allen's "Commercial Organic Analysis," 4th ed., recommends clarifying the solution by passing it through a layer of animal charcoal. This must *not* be done, since 0.5 gram of charcoal may adsorb 1 mg. of lead.<sup>1</sup>

6. The sulfates are used in preparing permanent standards since the color of ferric sulfate solutions does not vary with the temperature as much as solutions of the chloride. The ferric sulfate is conveniently prepared by dissolving ferrous sulfate, adding the requisite amount of sulfuric acid, a few cubic centimeters of nitric acid, and boiling until all the nitric oxide is expelled. A large excess of acid should be avoided.

7. In standardizing a set of permanent standards by mixing solu-

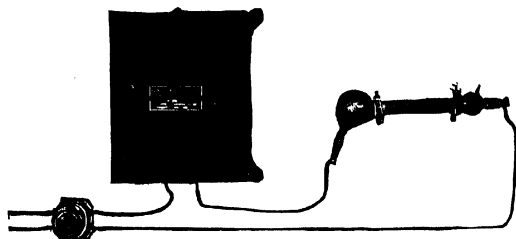


FIG. 49.—Cooper Hewitt Mercury Light. (Courtesy of D. Van Nostrand Co.)

tions of copper, cobalt, and ferric sulfates (or chlorides), the judgment of several pairs of eyes on the agreement, both in color tint and in depth of color, should be obtained before sealing the glass cylinders.

8. The Cooper Hewitt Mercury Light is excellent for the colorimetric determination of lead as the sulfide, where an artificial light is desired. "The yellow shades appear yellowish-green and may be matched more readily than the yellows obtained by daylight." Figure 49 illustrates the type of light recommended by Scott for this work.<sup>2</sup>

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#### METHOD B.—FORMATION OF PbS IN ALKALINE SOLUTION

##### Reagents.

1. Sodium thiosulfate solution, 0.1 N. Dissolve 25 grams of sodium thiosulfate,  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , in freshly boiled (and cooled) distilled water and dilute to a liter with boiled water.
2. Potassium cyanide solution. Dissolve 10 grams of potassium cyanide in water and dilute to 100 cc.
3. Ammonium hydroxide, sp. gr. 0.88.
4. Potassium sulfide solution, 10 per cent solution.
5. Standard lead solution. Dissolve 0.1599 gram of pure lead nitrate,  $\text{Pb}(\text{NO}_3)_2$ , or 0.1831 gram of pure lead acetate,  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ , in water, dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of lead.

**Procedure.**—A 35 cc. solution of the sample is prepared as directed in Method A, page 250. Add 2 cc. of 0.1 N sodium thiosulfate and heat the solution to incipient boiling. Remove the flame and let the solution stand about 5 minutes. Then add at once 1 cc. of 10 per cent potassium cyanide solution and a slight excess of concentrated ammonia. Boil gently until the solution becomes colorless, add 5 drops of 10 per cent potassium sulfide solution, mix gently, and compare at once with a standard lead sulfide suspension prepared under the same conditions and along with the sample.

##### Notes.

1. Ferrous compounds, but not ferric compounds, are converted into a colorless (in dilute solution) compound,  $\text{K}_4\text{Fe}(\text{CN})_6$ , which is not affected by alkaline sulfide. Theoretically, 1 mg. of iron requires 7 mg. of potassium cyanide for conversion to potassium ferrocyanide, but in practice about 25 mg. of the cyanide to 1 mg. of iron should be used.

The ferric iron, if present, must be reduced to ferrous iron and the

precipitation of  $\text{Fe}(\text{OH})_2$  must occur within the sphere of action of the KCN. The ferric iron is reduced by sodium thiosulfate according to the following reaction:



2. Sodium thiosulfate is rarely pure as sold commercially, but may easily be purified by recrystallization. The carbon dioxide absorbed from the air by water decomposes the salt, with the separation of sulfur in the form of a finely divided precipitate. Freshly boiled distilled water which has been cooled out of contact with air should be used in the preparation of the thiosulfate solution.

3. Where an artificial light is desired, the Cooper Hewitt Mercury Light is excellent. (See Note 8, page 252.)

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#### DETERMINATION OF LEAD AS THE CHROMATE

The method is based upon the yellow turbidity produced by adding potassium chromate to dilute solutions of lead salts.

##### Reagents.

1. Acetic acid, 6 N.
2. Potassium chromate, 10 per cent solution.
3. Standard lead solution. Dissolve 0.1831 gram of pure lead acetate,  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ , in water, add a few drops of acetic acid, make up to 100 cc., and mix thoroughly. Dilution of 10 cc. of this solution to a liter gives a solution containing 0.01 mg. of lead per cubic centimeter.

**Procedure.**—The sample should contain between 0.05 and 0.1 mg. The sample, adjusted, by dilution or evaporation, to within the proper concentration, is placed in a Nessler cylinder and diluted to 100 cc. Then add a drop of acetic acid and a drop of potassium chromate solution. If lead is present a turbidity appears slowly. After

standing half an hour the turbid solution is matched against a standard similarly prepared.

#### Notes.

1. A considerable excess of the chromate must be avoided.
2. The results are satisfactory for lead down to 0.02 mg., but is not recommended for lower concentrations.
3. The alkaline earth metals must be absent.

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#### DETERMINATION OF LEAD BY DIPHENYL CARBAZIDE

The method depends upon the formation of a violet-colored solution when *s*-diphenyl carbazide in glacial acetic acid (Cazeneuve's reagent) is added to a solution containing a small quantity of chromate. The intensity of the violet color is proportional to the amount of  $\text{H}_2\text{CrO}_4$  present. A measured quantity (an excess) of standard dichromate solution is added to the lead solution, the lead chromate removed by filtration, and the excess chromate determined in the filtrate.

#### Reagents.

1. Nitric acid, 6N.
2. Sodium sulfide, 5 per cent solution.
3. Sodium acetate. Use pure solid.
4. Diphenyl carbazide reagent (Cazeneuve's reagent). Dissolve 0.4 gram of pure *s*-diphenyl carbazide in 10 grams of glacial acetic acid.
5. Standard potassium dichromate solution. Dissolve 0.1420 gram of pure  $\text{K}_2\text{Cr}_2\text{O}_7$  in water, dilute to a liter, and mix thoroughly. One cubic centimeter of this solution is equivalent to 0.2 mg. of lead.

**Procedure.**—The lead is precipitated in the usual way as  $\text{PbS}$ , filtered, the precipitate washed with 5 per cent  $\text{Na}_2\text{S}$  solution, dissolved in boiling dilute  $\text{HNO}_3$ , evaporated to dryness, and the residue heated to  $130^\circ$ – $150^\circ$  C. for 1 hour. Cool, dissolve the residue in 10 cc. of water, evaporate again, cool, dissolve the residue in 5 cc. of water, and add a crystal of sodium acetate to remove the last trace of nitric acid.

The *neutral* lead solution thus formed is *added* to 25 cc. of the standard potassium dichromate solution. Now add 0.1 gram of pure, finely divided asbestos, shake the mixture for 10 minutes, filter, and bring the filtrate up to 100 cc. with the washings. Mix thoroughly the filtrate and test 5 cc. of it for excess of  $K_2Cr_2O_7$  by adding 2 drops of nitric acid and 1 drop of diphenyl carbazide reagent. If an excess of  $K_2Cr_2O_7$  was used to precipitate the lead, the solution turns violet. If an excess of  $K_2Cr_2O_7$  was not added, evaporate total filtrate to dryness, and add it to another 25 cc. of the standard potassium dichromate solution, etc., as before and make a second test with diphenyl carbazide reagent. The excess  $K_2Cr_2O_7$  is estimated by matching the intensity of the violet colored solution against that of a standard  $K_2Cr_2O_7$  solution similarly treated with the diphenyl carbazide reagent. The methods of balancing or diluting are convenient. By subtracting the excess of  $K_2Cr_2O_7$  solution from the *total* amount added the amount that reacted with the lead is obtained.

#### Notes.

1. The diphenyl carbazide reagent (Cazeneuve's reagent) gives a stable violet color to the solution when 1 part of Cr is present in 10 million parts of solution.

2. If the  $K_2Cr_2O_7$  solution were *added* to a neutral or even faintly acid solution of a lead salt, *basic* lead chromate of variable composition would be formed instead of neutral  $PbCrO_4$ .

3. This method is applicable to the determination of small quantities of lead in tinning baths, canned goods, and solders. [See Breteau and Fleury, J. pharm. chim. **10**, 265 (1914)]. About 1 gram of the sample is fused in a crucible with a mixture of 3 grams of  $Na_2CO_3$  and 3 grams of pure S. Dissolve the Sn with boiling water, take up the  $PbS$ ,  $CuS$ , and  $FeS$  with the smallest amount of HCl and Br water, boil, reduce the Fe completely by  $SO_2$ , and add an excess of both KCN and KOH. Add  $Na_2S$  to the solution. Only  $PbS$  will be precipitated. The  $PbS$  is filtered, washed with 5 per cent  $Na_2S$  solution, and estimated as given in the procedure above. The other constituents may be determined by the usual methods.

#### REFERENCES

1. Cazeneuve, Analyst, **25**, 331 (1900).
2. A. Moulin, Bull. soc. chim., **31**, (iii), 295 (1904).
3. P. Breteau and P. Fleury, J. pharm. chim., **10**, 265 (1914).
4. B. S. Evans, Analyst, **46**, 285 (1921).

### DETERMINATION OF LEAD BY HEMATIN

Moffatt and Spiro<sup>3</sup> describe a method for the colorimetric estimation of lead based upon the blue color obtained when 0.5 to 1 cc. of a hematin (0.5 gram per liter) solution is added to solutions containing small amounts of lead. The method is applicable to the determination of lead in drinking water. One part of lead in two million parts of solution can be recognized. Cu, Zn, and Fe should be absent.

### DETERMINATION OF LEAD BY ANILINE

Morgan<sup>4</sup> gives a simple method for the determination, in a semi-quantitative way, of lead dioxide in the presence of litharge based upon the oxidation of aniline to aniline purple. The method has been used successfully with rubber work where it is important to know the amount of oxidizing agent present. With slight alterations it is applicable to the determination of oxidizing agents in general where they occur as impurity with non-oxidizing agents.

**Procedure.**—About a 5 gram sample of litharge is boiled one minute with a solution of 2 grams of aniline hydrochloride dissolved in 10 cc. of water and 5 cc. of concentrated hydrochloric acid. The solution is cooled (to separate any  $PbCl_2$ ), filtered, and the filtrate compared with standards made by adding known amounts of  $PbO_2$  to the aniline hydrochloride solution.

**Note.**—Since the color reaction depends upon the oxidizing power of the dioxide only, it is not necessary to convert the litharge into lead chloride.

### DETERMINATION OF LEAD BY SODIUM BISULFITE

This method<sup>5</sup> is based upon the milky turbidity produced when sodium bisulfite is added to a solution containing a small amount of lead. The reaction is said to be sensitive to 1 part of lead in 20 million parts of solution.

**Procedure.**—Fifty cubic centimeters of the sample are mixed with 50 cc. of a 2 per cent solution of  $NaHSO_3$ . If a turbidity forms in a few minutes the lead content is about 1 part in a million.

**Note.**—Cu, Ag, Ni, Fe, Al, Mg, and Ca have no influence on the reaction; Ba and Sn (especially stannous tin) should be absent.

<sup>3</sup> Chem. Ztg., **31**, 639 (1907).

<sup>4</sup> J. Ind. Eng. Chem., **11**, 1055 (1919).

<sup>5</sup> V. N. Ivanov, Chem. Ztg., **38**, 450.



## DETERMINATION OF LEAD IN URINE AND FECES

METHOD OF KEHOE, EDGAR, THAMANN AND SAUNDERS<sup>6</sup>

This method was developed in the course of an extensive investigation of the excretion of lead in the human subject. It is a modification of Fairhall's chromate titration method,<sup>7</sup> the final analysis being made colorimetrically by adding a 1 per cent solution of pure *s*-diphenyl carbazide in glacial acetic acid to an aliquot portion of the lead chromate solution obtained from the sample. The aliquot should not contain more than 0.4 to 0.5 mg. of lead, the best results being obtained with about 0.2 mg.

**Reagents.** (See Note 2)

1. Hydrochloric acid, sp. gr. 1.19.
2. Hydrochloric acid, 1 : 1.
3. Hydrochloric acid, 1 : 2.
4. Hydrochloric acid, 10 per cent (by volume).
5. Nitric acid, sp. gr. 1.42.
6. Nitric acid, 1 : 1.
7. Acetic acid, 5 per cent.
8. Ammonium hydroxide, sp. gr. 0.90.
9. Sodium hydroxide, 25 per cent. Use sodium hydroxide free from iron and aluminum.
10. Hydrogen sulfide. Use thoroughly washed hydrogen sulfide gas. See p. 240.
11. Hydrogen sulfide water. Use a freshly prepared saturated solution containing 1 cc. of concentrated hydrochloric acid per liter.
12. Potassium chromate, 1 per cent.
13. Methyl red, 0.1 per cent. Dissolve 0.1 gram of methyl red in 100 cc. of 95 per cent alcohol.
14. Phenolphthalein. Use a slightly alkaline water solution.
15. *s*-Diphenyl carbazide reagent. Use a 1 per cent solution of chemically pure *s*-diphenyl carbazide in glacial acetic acid.
16. Whatman filter paper No. 40. 7 cm. and 12.5 cm.
17. Standard lead chromate solution. Dissolve 0.1560 gram of pure lead chromate in 200 cc. of 10 per cent hydrochloric acid, dilute to a liter, and thoroughly mix. Check the solution colorimetrically

<sup>6</sup> J. Am. Med. Assoc., 87, 2081 (1926).

<sup>7</sup> J. Ind. Hyg., 4, 9, (1922).

against standard potassium dichromate solution. One cubic centimeter of the standard lead solution contains 0.1 mg. of lead.

**Procedure for Urine.**—As soon as the sample of urine is received, measure its volume and make ammoniacal. After standing for at least 12 hours, the sample is filtered on 18 cm. folded filter paper, care being taken that all of the precipitate is transferred to the filter. Without being washed, the filter paper is transferred to a silica or porcelain dish and, after being dried on a steam-plate, is ignited in an electric muffle furnace at a temperature not exceeding 600° C. When the organic matter has been completely destroyed, the residue is treated as follows: The ash is moistened carefully with distilled water and 5 cc. of concentrated hydrochloric acid are added, together with enough water to make the volume about 25 cc. The mixture is digested on a steam-plate and then filtered into a 400 cc. beaker, any residue being washed six times alternately with hot 10 per cent hydrochloric acid and hot water. (The filtrate in the beaker should contain all the lead. The residue is usually insignificant and may be disregarded.)

The solution is neutralized by adding 25 per cent sodium hydroxide solution until a faint turbidity persists. If too much sodium hydroxide is added, the solution is treated with hydrochloric acid until it is perfectly clear and sodium hydroxide again added until a faint turbidity appears. In some cases addition of sodium hydroxide causes no turbidity, even after the sample is distinctly alkaline to methyl red. To avoid excess of alkali in these cases methyl red is added to all samples. When the samples contain sufficient calcium phosphate to give the turbidity mentioned the reaction of the indicator is ignored. In all other cases the sample is made just alkaline.

The solution is diluted to 300 cc. and after cooling, if necessary, is treated with hydrogen sulfide gas for one hour and allowed to stand overnight. The mixture is filtered on a 12.5 cm. Whatman No. 40 filter paper, any precipitate being transferred to the paper by means of freshly prepared hydrogen sulfide water acidified with 1 cc. of concentrated hydrochloric acid per liter. The paper and its contents are transferred to the beaker in which the sulfide precipitation was made. The sides of the beaker are washed down with 25 cc. of warm (1 : 1) hydrochloric acid containing 10 drops of concentrated nitric acid. The mixture is digested with acid until all sulfides are dissolved and the paper thoroughly white. The solution is diluted to 50 cc. with hot water and is filtered, the residue being washed fifteen times with hot

water. To the filtrate (about 400 cc.) is added 4 drops of a solution of methyl red, to which it is made just alkaline with 25 per cent sodium hydroxide, then just acid with (1 : 2) hydrochloric acid, after which 1 cc. of (1 : 2) hydrochloric acid is added in excess. This is diluted to 300 cc. and after cooling is treated with hydrogen sulfide gas for one hour and allowed to stand overnight.

Filter the mixture on a 12.5 cm. Whatman No. 40 filter paper, the precipitate being transferred to the paper by means of freshly prepared hydrogen sulfide water containing 1 cc. of concentrated hydrochloric acid per liter. The beaker is washed carefully with this wash water and the filter is washed ten times. The sulfides are dissolved from the paper with from 10 to 20 cc. of hot (1 : 1) nitric acid, the solution being caught in the beaker in which the precipitation was made. The paper is washed fifteen times with hot water. The sides of the beaker and the inside and outside of the tube from the hydrogen sulfide generator are washed with hot (1 : 1) nitric acid. The solution is evaporated to 5 cc., diluted to 25 cc. with hot water, and filtered through a 7 cm. filter paper into a 150 cc. beaker. The beaker is carefully washed with hot water and the filter paper is washed fifteen times with hot water. The filtrate and washings are evaporated to 25 cc. and neutralized with 25 per cent sodium hydroxide, *free* from iron and aluminum, a slightly alkaline water solution of phenolphthalein being used as indicator.

Make the solution faintly pink by adding alkali, add 5 per cent acetic acid until the color is just discharged, and then add 2 cc. of the acid in excess. Bring the solution to boiling and add 1 cc. of 1 per cent potassium chromate solution. Place the mixture on a steam-bath for one hour and allow it to stand in a warm place overnight. It is then brought to boiling, filtered on a 7 cm. *ashless* filter paper, the beaker being washed carefully with hot water, and the filter paper being washed fifteen times with hot water. Any residue is dissolved in from 5 to 15 cc. of cold (1 : 1) hydrochloric acid, the paper being washed at once with cold water, the solution and washings being caught in the beaker in which the chromate precipitation was made. The sides of the beaker and the stirring rod are washed with cold (1 : 1) hydrochloric acid.

For the analysis an aliquot portion of the solution of lead chromate obtained above is taken, which should contain not more than 0.40 to 0.50 mg. of lead, the best results being obtained with about 0.20 mg.

The sample is diluted to about 100 cc. in a Nessler tube, and an equal volume of water to which has been added a quantity of hydrochloric acid equivalent to that in the sample taken for analysis is put into another Nessler tube. Two cubic centimeters of diphenyl carbazide reagent are added to each tube. From the color which develops in the tube containing the sample, a rough estimate is made of the quantity of lead present, and an amount of standard lead chromate slightly smaller than this is added to the tube that is to be the standard. The colors of the two solutions are compared, and standard lead chromate is added drop by drop to the standard tube until an exact match is obtained.

**Procedure for Feces.**—Transfer the sample of feces to a silica dish and dry on a steam-plate to “apparent dryness.” No attempt need be made to bring the feces to constant weight. The sample of dried feces is ashed in a silica dish in an electric muffle at from 500° to 600° C. When the organic matter is apparently destroyed, the residue is moistened with water, acidified with hydrochloric acid and digested on the steam-plate for some time. The mixture is then filtered, the residue washed with hot dilute hydrochloric acid and hot water, and the filter paper and its contents returned to the silica dish in which the original ashing was done. After being dried on the steam-plate, the dish and its contents are returned to the furnace and ashed again at from 500° to 600° C. The residue is treated with water and hydrochloric acid as before and, after being digested on the steam-plate, is filtered, the filtrate being added to the first filtrate. The residue may contain traces of lead, and may be brought into solution with hydrofluoric acid and added to the main solution. However, the experience of Kehoe *et al.*<sup>8</sup> showed that after careful washing the residue will not contain an appreciable quantity of lead and may be neglected. The solution obtained from the feces is diluted and neutralized with 25 per cent sodium hydroxide, exactly as described above in the procedure for urine.

The remainder of the analysis is exactly like that described for the analysis of urine, except that the second hydrogen sulfide precipitation is repeated, a third precipitation being made exactly as the second. The precipitation as chromate and the colorimetric determination are made just as in the case of urine.

<sup>8</sup> *Loc. cit.*

**Notes.**

1. Obviously a standard colorimeter may be employed in place of the Nessler tubes. In any case, it is essential that the amounts of lead in the standard and the unknown be as nearly identical as possible.

2. Lead-free reagents must be employed throughout. It is possible to purchase them in the market, but they must be carefully tested for lead before being used. Pyrex glass beakers, flasks, wash bottles, etc., and soda glass funnels are satisfactory. For ashing feces and urine 400 cc. opaque silica dishes may be used. The containers for the samples may be of soft glass. The following table shows the amount of lead in materials used in tests by Kehoe *et al.*:<sup>9</sup>

TABLE XVII

Article	Quantity	Lead in Mg.
Pyrex.....	1.0205 grams	0.22
Silica dish.....	3.5415 grams	<i>Nil</i>
Soft glass funnel.....	1.4395 grams	0.13
Mason jar.....	1.2775 grams	0.24
Glass jug.....	1.0555 grams	0.02
Concentrated hydrochloric acid, specific gravity 1.19.....	100 cc.	<i>Nil</i>
Concentrated nitric acid, specific gravity 1.42.....	50 cc.	<i>Nil</i>
Sodium hydroxide, 25 per cent.....	200 cc.	<i>Nil</i>
Ammonium hydroxide, 28 per cent.....	50 cc.	<i>Nil</i>
Whatman filter paper No. 40.....	25-12.5 cm.	0.03
Munktell filter paper No. 1 F.....	25-7 cm.	<i>Nil</i>

The data in the table show that the glassware employed contained traces of lead when considerable weights of it were decomposed with hydrofluoric acid and analyzed; but it appears that no appreciable lead can enter the samples from this source, since the amount of glass actually dissolved is extremely small. This is borne out by the fact that numerous blank determinations on reagents were obtained when the same glass and the same technique were used.

3. The ordinary technique of good analytic work must be observed most scrupulously if satisfactory results are to be obtained in analyzing organic material for small amounts of lead. Precautions as to the cleanliness of the laboratory and the apparatus are essential. If

<sup>9</sup> *Loc. cit.*

practical, no other work than the lead analyses should be carried out in the laboratory employed for the purpose.

4. Fairhall<sup>10</sup> has shown that ammonia precipitates practically all of the lead from urine, it being carried down with the alkaline-earth phosphates. The procedure is much more convenient than that involving the evaporation of the urine, and Kehoe *et al.*<sup>11</sup> have verified its adequacy. If it is in error at all, it is in the direction of giving slightly low results.

<sup>10</sup> J. Biol. Chem., **60**, 485 (1924).

<sup>11</sup> *Loc. cit.*

## CHAPTER XXII

### MAGNESIUM

#### DETERMINATION OF MAGNESIUM BY THE BELL-DOISY REACTION

THIS method is based upon the fact that various phenols do not reduce  $\text{MoO}_3$  but do reduce phosphomolybdic acid. Hydroquinone is the phenol selected for use in the method. The magnesium is separated as magnesium ammonium phosphate and the latter converted into phosphomolybdate, which in turn is treated with the hydroquinone and carbonate-sulfite solutions of Bell and Doisy,<sup>1</sup> and the resulting color matched against a standard.

The method is applicable to the determination of small quantities of magnesium in urine, blood, tissue extracts, and incinerations.

#### Reagents.

1. Hydrochloric acid, 0.1 N.
2. Ammonium hydroxide, 10 per cent.
3. Di-ammonium hydrogen phosphate.
4. Alcohol, 90-95 per cent.
5. Ammonium molybdate. Dissolve 50 grams of ammonium molybdate,  $(\text{NH}_4)_2 \text{MoO}_4$ , in a liter of  $\text{N H}_2\text{SO}_4$ . Five cubic centimeters of this solution should give no color when treated with 5 cc. of (6) and, after 5 minutes, with 25 cc. of (7). If the molybdate contains phosphate it may be purified according to the method of Bell and Doisy. (See Note 1.)
6. Hydroquinone. Dissolve 20 grams in a liter and add 1 cc. of concentrated sulfuric acid.
7. Carbonate-sulfite solution. To 400 cc. of 20 per cent sodium carbonate add 15 grams of sodium sulfite dissolved in 100 cc. of water and filter.

<sup>1</sup> R. D. Bell and E. A. Doisy, *J. Biol. Chem.*, **44**, 55 (1920); B. Kramer and F. F. Tisdall, *ibid.*, **48**, 1, 223 (1921); *Bull. Johns Hopkins Hosp.*, **32**, 44 (1921); A. P. Briggs, *J. Biol. Chem.*, **52**, 349 (1922); F. S. Hammett and E. T. Adams, *ibid.*, **52**, 211 (1922); *ibid.*, **54**, 565 (1922).

8. Standard phosphate solution. Dissolve 4.388 grams of potassium di-hydrogen phosphate,  $\text{KH}_2\text{PO}_4$ , in water, dilute to a liter and thoroughly mix. Preserve with  $\text{CHCl}_3$ . One cubic centimeter contains 1 mg. of P. The weight of P found multiplied by 0.7838 gives the amount of Mg present in the sample.

**Procedure.**—After precipitation of the calcium with ammonium oxalate according to Kramer and Tisdall,<sup>2</sup> pipette off 5 or 10 cc. of the clear supernatant liquid, put into a 25 cc. centrifuge tube, add, drop by drop, 1 cc. of di-ammonium hydrogen phosphate solution, and then 2 cc. of ammonium hydroxide, also added drop by drop. Thoroughly scratch the sides of the tube and allow the mixture to stand overnight. The precipitate is then centrifuged off, washed twice with 10 per cent ammonium hydroxide and once with ammoniacal alcohol, dried at  $70^\circ \text{C}$ ., and dissolved in 10 cc. of 0.1 N hydrochloric acid in the tube. Transfer the solution to a 25 cc. volumetric flask. In a second 25 cc. volumetric flask place 5 cc. (0.05 mg. P) of the standard potassium di-hydrogen phosphate solution. To both flasks add 5 cc. of distilled water (phosphate-free), 1 cc. of the molybdate solution, and 2 cc. of the hydroquinone solution. After standing 5 minutes, add to each flask 10 cc. of the carbonate-sulfite solution. Dilute the solutions to 25 cc., mix thoroughly, and after 5 or 10 minutes compare in a colorimeter. The amount of P found multiplied by 0.7838 gives the quantity of Mg in the sample removed from the supernatant liquid from the calcium precipitation.

#### Notes.

1. If the ammonium molybdate contains phosphate it may be purified by dissolving 150 grams in a liter of water and adding the solution to a liter of a solution containing 375 cc. of concentrated nitric acid (sp. gr. 1.42), mixing, and then adding 200 grams of ammonium nitrate and allowing to stand for several days in a warm place. The precipitate is filtered off and the filtrate mixed with 2 volumes of alcohol and enough ammonium hydroxide added to leave the solution only slightly acid to litmus. The ammonium molybdate is filtered off in a few minutes, washed with 50 per cent alcohol, and dried.

2. The magnesium determinations have a tendency to be a little low. This is due either to incomplete precipitation or to solution

<sup>2</sup> *Loc. cit.*



during washing, but with care the loss can be held to within about 3 per cent.<sup>3</sup>

3. The carbonate-sulfite solution should not be over 2 weeks old and should have been kept in a tightly stoppered bottle. Fading of the color, which is due to oxidation of the sulfite to sulfate, is thus avoided.

4. The separation of magnesium ammonium phosphate by centrifugation is preferable to filtration because the best acid-washed asbestos, or pulp made from the best grade of filter paper, contains sufficient phosphate, or other substances capable of giving the Bell-Doisy reaction, to yield a well-defined colorimetric test.<sup>4</sup>

**DETERMINATION OF MAGNESIUM BY SEPARATION AS MAGNESIUM  
AMMONIUM PHOSPHATE AND ESTIMATION OF THE  
PHOSPHATE AS PHOSPHOMOLYBDATE**

The method<sup>5</sup> depends upon the quantitative separation of the magnesium by precipitation as magnesium ammonium phosphate, dissolving the precipitate in nitric acid, and estimating the phosphate by comparing with a standard the yellow color developed upon adding ammonium molybdate solution. The color is proportional to the amount of phosphate present, and hence to the magnesium content. The method is applicable to the estimation of magnesium in water or in various substances containing a small amount of magnesium.

**Reagents.**

1. Nitric acid, sp. gr. 1.07.
2. Ammonium hydroxide, 6 N.
3. Ammonium hydroxide wash solution. Dilute 1 part of strong ammonia (sp. gr. 0.90) with 9 parts of water. The ammonia must be free from silica and, hence, only redistilled ammonium hydroxide should be used.
4. Ammonium molybdate solution. Fifty grams of the pure salt are dissolved and the solution diluted to a liter.
5. Standard phosphate solution. Dissolve 0.5043 gram of pure, freshly crystallized di-sodium hydrogen phosphate,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , in water, add 100 cc. of nitric acid (sp. gr. 1.07), dilute to a liter, and

<sup>3</sup> F. S. Hammett and E. T. Adams, *J. Biol. Chem.*, **52**, 211 (1922).

<sup>4</sup> F. S. Hammett and E. T. Adams, *J. Biol. Chem.*, **54**, 565 (1922).

<sup>5</sup> O. Schreiner and W. S. Ferris, *J. Am. Chem. Soc.*, **26**, 961 (1904).

thoroughly mix. One cubic centimeter of this solution is equivalent to 0.1 mg. of  $P_2O_5$  or 0.0342 mg. of Mg.

6. Standard colorimetric solution. Dilute 10 cc. of the standard phosphate solution (3) to about 80 cc., add 9 cc. of nitric acid (2) and 8 cc. of ammonium molybdate solution (1), and dilute to 100 cc. Mix thoroughly and allow to stand 20 minutes. One cubic centimeter of this solution is equivalent to 0.01 mg.  $P_2O_5$  or 0.00342 mg. magnesium, i.e., the amount of phosphorus as  $P_2O_5$  is multiplied by the factor 0.342 to give the amount of magnesium present. The factor to represent the result in terms of MgO is 0.568.

7. Ammonium oxalate. Saturated solution.

8. Phosphate reagent. Dissolve 17.4 grams of di-potassium hydrogen phosphate,  $K_2HPO_4$ , and 100 grams of ammonium chloride in about 900 cc. of water, add 50 cc. of ammonia (sp. gr. 0.90), and dilute to a liter. One cubic centimeter of this solution will precipitate 2.4 mg. of magnesium.

9. Filter paper. Use only silica-free paper.

**Procedure.**—Measure out a sample which contains between 0.00003 and 0.0001 gram of magnesium and dissolve if a solid. Make the solution faintly ammoniacal by adding 1 drop excess of 6 N ammonium hydroxide and add 2 or 3 drops of the ammonium oxalate solution. Evaporate to dryness on a water-bath, cool, and add 1 cc. of the phosphate solution. Thoroughly stir the precipitate and allow to stand 2 or 3 hours. Then wash down the sides of the dish with 5 cc. of ammonium hydroxide wash solution and filter off, on a small filter, the magnesium ammonium phosphate. Repeat washing the dish with successive small amounts of the wash liquid until all the precipitate has been transferred to the filter, finally washing down the filter until the filtrate measures about 50 cc. Wash the dish once with about 5 cc. of cold water, allowing the water to run through the filter in such a way as to wash it. Reject the washings and place a small beaker under the funnel. Add 5 cc. of nitric acid to the evaporating dish, thoroughly spread it so as to insure complete removal of any precipitate that may have remained on the sides of the dish and then pour the solution through the filter in such a way as to wet the whole of it. Wash the dish four or five times with hot water (about 5 cc. each time) and continue washing the filter until the filtrate increases to about 45 cc. Cool the filtrate, add 4 cc. of the ammonium molybdate solution, dilute to 50 cc., mix, let stand 20 minutes, and compare the color with that

of the standard phosphate solution,\* by the balancing or dilution method.

**Notes.**

1. The yellow color which develops is at its maximum intensity after 20 minutes and, hence, the solution must be allowed to stand this period before making the comparison. If the color is too strong for direct comparison with the standard, an aliquot part is used.

2. Great care must be taken to add enough of the molybdate reagents. The 5 cc. of nitric acid and 4 cc. of ammonium molybdate solution given in the procedure are sufficient only up to about 0.0003 gram of magnesium. When a second portion of these reagents is required (as indicated by the amount of precipitate or development of color), the solution is diluted with water at the same time so as to keep the concentration of the reagents the same, i.e., 5 cc.  $\text{HNO}_3$  and 4 cc. of molybdate solution per 50 cc. of the solution.

3. The 2 or 3 drops of ammonium oxalate solution are added before adding the phosphate reagent in order to prevent the calcium precipitating as calcium phosphate.

4. Silica gives a yellow color with the molybdate reagent as does phosphate and, hence, must be removed. In fact, the color produced by the silicomolybdates is even more intense than that of the phosphomolybdates.<sup>6</sup> Since an alkaline liquid is used throughout the procedure, dissolved silica will always be present and is removed in the rejected washings. The last washing must be made with pure water on account of traces of dissolved silica always present in ammonia water. Use only freshly distilled ammonia for preparing the wash solution.

5. If the sample is a solid, it is dissolved in the smallest amount of nitric or hydrochloric acid possible and the excess acid removed by evaporation to dryness. The residue is dissolved in water and the procedure continued in the usual way. Should the sample be a liquid, say a potable water, it may be necessary to concentrate by evaporation. The magnesium content of the sample should be between 0.03 and 0.1 mg.

6. The standard phosphate solution is acidified with nitric acid in order to lessen contamination with silica from the glass bottle.

7. Any coloring matter in the sample is entirely removed or destroyed during the procedure and, hence, has no influence on the final color comparison.

\* O. Schreiner and B. E. Brown, J. Am. Chem. Soc., **26**, 1463 (1904).

8. The method is applicable to natural water or to soil and plant extracts. The presence of other salts, in amounts likely to be present, has no influence on the accuracy of the method beyond the possible error of reading the colorimeter.

The following results show the limits of accuracy of the method. They are taken from a list of 22 determinations made by Schreiner and Ferris,<sup>7</sup> and are representative. All the solutions contained (in addition to magnesium) calcium and potassium sulfates, nitrates, and chlorides.

TABLE XVIII

Milligrams Mg		Parts Mg per Million of Solution	
Present	Found	Present	Found
1.265	1.311	25.30	26.22
0.949	0.984	18.98	19.68
0.632	0.610	12.64	12.20
0.474	0.490	9.48	9.80
0.316	0.313	6.32	6.26
0.190	0.190	3.80	3.80
0.063	0.074	1.26	1.48
0.025	0.045	0.50	0.90

Schreiner and Ferris also tested the efficiency of the procedure in the removal of silica. All the solutions contained more than 5 parts of silica per million of solution, as well as salts of calcium and potassium. The following are representative results of S. and F.:

TABLE XIX

Milligrams Mg		Parts Mg per Million of Solution	
Present	Found	Present	Found
0.316	0.316	6.32	6.32
0.158	0.147	3.16	2.94
0.079	0.088	1.58	1.76
0.040	0.055	0.80	1.10

<sup>7</sup> *Loc. cit.*

**DETERMINATION OF MAGNESIUM AS THE OLEATE**

The method<sup>8</sup> depends upon the formation of a pale yellow color due to a colloidal suspension of magnesium oleate. Since calcium produces a similar color, it must be removed.

**Reagents.**

1. Ammonium chloride reagent. Dissolve 100 grams of  $\text{NH}_4\text{Cl}$  and 9 grams of  $\text{NH}_3$  and dilute to a liter.
2. Oleic acid reagent. Dissolve 2 grams of oleic acid and 0.5 gram of  $\text{KOH}$  in 600 cc. of 95 per cent alcohol and 400 cc. of water.

**Procedure.**—A sample is taken which contains 0.008 to 0.1 mg. of  $\text{MgO}$ . Dissolve, if a solid, and treat the solution with 2 cc. of the ammonium chloride reagent and 1 cc. of the oleic acid reagent. Dilute the solution to 50 cc., allow to stand 2 hours, and then compare the color with that obtained with known quantities of magnesium.

<sup>8</sup> A. Grégoire and T. Sola, Bull. soc. chim. Belg., **32**, 131 (1923).

## CHAPTER XXIII

### MANGANESE

#### DETERMINATION OF MANGANESE BY OXIDATION WITH PERSULFATE

THE method is based upon the oxidation of manganese to permanganate, the permanganate ions imparting a pink to red color to the solution, depending upon the concentration.<sup>1</sup>

##### Reagents.

1. Nitric acid or sulfuric acid, 6 N.
2. Ammonium or potassium persulfate, C. P. solid.
3. Silver nitrate, 0.2 N.
4. Standard permanganate solution. The use of an old standard permanganate solution, whose iron factor is known, is recommended. To obtain the manganese factor, multiply the iron factor of the solution by 0.2952.

If a new standard is to be made up, dissolve 0.1438 gram of pure potassium permanganate in water and dilute to 100 cc. Thoroughly mix and dilute 10 cc. of the solution to one liter, after adding 10 cc. of dilute nitric acid. This solution contains 0.05 mg. of manganese per cubic centimeter. All water used in dissolving and diluting the potassium permanganate in the preparation of the standard should be distilled from alkaline permanganate and redistilled.

**Procedure.**—A sample is weighed out according to its manganese content (see Note 1), dissolved in dilute nitric or sulfuric acid, and any silica present is filtered off and washed with a minimum of water. Add 10 cc. of the silver nitrate solution and crystals of ammonium or potassium persulfate sufficient to oxidize all the manganese to permanganate. The solution contained in a comparison cylinder or a 100 cc. volumetric flask is placed in a tall beaker of boiling water and allowed to remain until the maximum intensity of pink has developed. The solution is then cooled to room temperature under the water tap,

<sup>1</sup> H. Marshall, *Chem. News*, **63**, 76 (1901).

diluted to the mark, thoroughly mixed, and the whole of it, or an aliquot part, compared against a standard permanganate solution by the method of duplication, balancing, or dilution. For the balancing or dilution method, 100 cc. of the standard may be diluted to 500 cc. with freshly boiled and cooled water. This gives a standard solution containing 0.01 mg. of manganese per cubic centimeter. (See Note 8.)

### Notes.

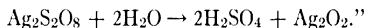
1. For a manganese content between 0.02 and 0.25 per cent, use a 0.5 gram sample; for a content between 0.25 and 4 per cent, use a 0.25 gram sample. When more than 4 per cent of manganese is present, a 0.1 gram sample is taken, or an aliquot part of its solution.

2. Chromium is practically the only metal that interferes, and it only when present in considerable excess of the permanganate. When a large amount of iron is present, as in steel analysis, phosphoric acid is added to prevent its precipitation or the formation of a yellow color.

3. The small quantity of silver nitrate is added as a catalytic agent in the oxidation by the persulfate. Without silver nitrate the oxidation is very slow and uncertain. Marshall<sup>2</sup> discovered this catalyzing action of silver nitrate and suggested the following mechanism. The ammonium or potassium persulfate solution undergoes the following decomposition in the presence of a small quantity of silver nitrate:



“Such actions seem to depend on the formation and decomposition of silver peroxide, which is probably produced by action of water on silver persulfate:



4. In case free chlorine or hydrochloric acid is present in the solution of sample, an excess of silver nitrate must be added to precipitate the chlorine, and the additional quantity added as the catalyzer. A trace of chloride would give an opalescent solution and hence would interfere with the comparison.

5. If chromium is present in too large a quantity, an excess of ammonium hydroxide is added to the solution after oxidation with persulfate. This precipitates the manganese as hydrated oxide, together with silver hydroxide and a few other metals. The chro-

<sup>2</sup> *Loc. cit.*

mium, however, remains in solution as chromate. The precipitate is filtered off, dissolved in nitric acid, ammonium or potassium persulfate added, and the solution placed in a beaker of warm water as described in the Procedure.

6. As little as 0.002 mg. of manganese per cubic centimeter can be detected by the distinct pink coloration the permanganate imparts to the solution.

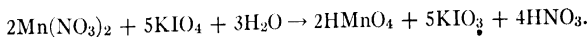
7. The persulfate method is erratic in the absence of the silver nitrate (Ag ions) catalyzer, and, even with the catalyzer, fairly close control of the concentration of acid and of manganese and of the time of heating is necessary to obtain accurate results and to prevent the precipitation of manganese dioxide. Oxidation is sometimes incomplete; the true permanganate color is not always obtained, and the color frequently fades after a short time.

8. If the highest degree of accuracy is desired, the standard permanganate solution should be reduced to manganous sulfate with sulfur dioxide, the excess expelled by boiling, the solution cooled and diluted to a convenient volume with redistilled water. A measured volume of this solution is then oxidized by persulfate under conditions identical with those used in oxidizing the sample. The tint of both sample solution and standard should then be the same and, hence, should permit greater accuracy in the comparison.

#### DETERMINATION OF MANGANESE BY OXIDATION WITH PERIODATE

##### METHOD OF WILLARD AND GREATHOUSE<sup>3</sup>

This method depends upon the oxidation in acid solution of manganous salts to permanganate by means of periodate, the reaction being represented by the following equation:



Only a small excess of periodate is required, but the reaction must be carried out in an acid solution sufficiently concentrated to prevent precipitation of the manganese.

The method is especially adapted for the determination of manganese in water, soil, ores, iron, and steels. It is free from all the faults of other methods and yields results of a high degree of accuracy.

<sup>3</sup> J. Am. Chem. Soc., **39**, 2366 (1917).



**Reagents.**

1. Sulfuric acid, sp. gr. 1.84; nitric acid, sp. gr. 1.42; or phosphoric acid, sp. gr. 1.70.

2. Potassium or sodium periodate. Solid.

3. Standard manganous sulfate solution. This solution is prepared by reducing an accurately measured volume of standard permanganate solution with sulfur dioxide, boiling off the excess of sulfur dioxide, cooling, and diluting to the proper volume to give a manganese concentration of 0.1 mg. per cubic centimeter. The permanganate solution is prepared by dissolving the best grade of "analyzed" potassium permanganate in water that has been distilled from alkaline permanganate and redistilled. This gives a very stable solution which is then carefully standardized against sodium oxalate, iron, ferrous sulfate, or ferrous ammonium sulfate of known purity.

If the highest degree of accuracy is not required, the standard permanganate solution may be diluted to give a manganese content of 0.02 mg. per cubic centimeter and this solution employed directly for the comparison.

**Procedure.**—The following is the "General Procedure" given by Willard and Greathouse,<sup>4</sup> who were the first to propose the use of periodate for the oxidation of manganous salts to permanganate in the colorimetric estimation of manganese:

The material to be analyzed is brought into a solution containing in 100 cc. at least 10 to 15 cc. concentrated sulfuric, 20 cc. of nitric or 5 to 10 cc. of syrupy phosphoric acid, or mixtures of two or more acids. The solution should previously have been freed from reducing agents by boiling with nitric acid, a little persulfate being added if carbon compounds are present, as with steel; if chloride is present it should be evaporated with nitric and sulfuric acids to fumes of the latter. 0.2 to 0.4 gram of  $\text{KIO}_4$  or  $\text{NaIO}_4$  is added, or an equivalent amount of  $\text{Na}_3\text{H}_2\text{IO}_6$ , the solution boiled for a minute, kept hot 5 to 10 minutes, cooled, diluted to the proper volume, and compared with a standard of known manganese content similarly prepared. When ready for comparison the solution should not contain much more than 1 mg. of manganese per 50 cc., otherwise the color will be too dark.

In the presence of considerable iron, either sulfuric or phosphoric acid must be present, since ferric periodate is insoluble in fairly concentrated nitric acid but readily soluble in the other acids. A very

<sup>4</sup> *Loc. cit.*

large concentration of acid does no harm in any case, neither does a longer time of heating.

Color comparisons may be made by any of the usual methods.

The following notes are based upon the experiments of Willard and Greathouse.<sup>5</sup>

#### Notes.

1. The periodate method is free from all the faults of other methods and yields results of a high degree of accuracy. (See Notes 4 and 7 under the persulfate method.)

2. Solutions of manganous salts oxidized by periodate have exactly the same color as pure permanganate solution.

3. Occasionally difficulty is caused by variation in tint with sulfuric and phosphoric acid solutions, arising from the brownish color of the carbon compounds after solution in nitric acid. This effect is especially noticeable when the carbon content is 0.8 per cent or more. To overcome this, the sample is dissolved in a mixture of 15 cc. nitric acid, 15 cc. water, and 15 cc. phosphoric or sulfuric acid, 1 gram of ammonium persulfate added and the solution decolorized by boiling 3 to 5 minutes. Analyses made by Willard and Greathouse, using steels with carbon content between 0.8 and 1.0 per cent, were accurate to within about  $\pm 0.003$  per cent of the manganese present. The manganese content varied in the different steels between 0.3 and 0.8 per cent.

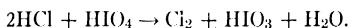
4. Determinations in which  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_3\text{PO}_4$  were used separately and in various concentrations were made by Willard and Greathouse. In all cases the only effect of increasing the concentration of acid above the minimum required to prevent precipitation is to increase the rate of oxidation of the manganese. This minimum concentration of acid increases with the concentration of manganese, and varies with different acids, being lowest for phosphoric acid. The well-known stability of manganic phosphates probably accounts for the non-appearance of precipitates of iodates or periodates of manganese even in solutions of very low acid concentration.

5. The only effect of varying the concentration of periodate is a slight increase in the speed of the reaction as the concentration of periodate is increased. Willard and Greathouse found complete oxidation was obtained in a manganese solution containing only 0.1 gram

<sup>5</sup> *Loc. cit.*

KIO<sub>4</sub>. The theoretical requirement was 0.052 gram KIO<sub>4</sub>. They also obtained complete oxidation of 0.06 gram of manganese in 100 cc. of solution containing 2 grams KIO<sub>4</sub>. This cannot be accomplished by any of the other methods. Even larger amounts of manganese can be oxidized, but such solutions are so deeply colored as to be of no practical value.

6. Periodic acid readily oxidizes hydrochloric acid to chlorine according to the equation:



The complete removal of chloride is obtained by heating the solution of sample with excess of periodate until the odor of chlorine has disappeared.

7. The presence of ammonium salts does not affect the intensity of the color, the tint of the solution, or the speed of oxidation of manganese solutions oxidized to permanganate by periodate.

8. "A remarkable feature of the solutions oxidized by periodate is their great stability when a slight excess of the reagent is present. Such a solution, kept for 3 months in a stoppered flask, when compared with a similar solution freshly oxidized showed no change whatever. This makes it possible to leave the standard solution in the colorimeter, renewing it only occasionally instead of preparing a fresh solution each time, as is necessary when persulfate is used." (Willard and Greathouse, *loc. cit.*)

9. The common metals do not interfere in this method except insofar as they themselves impart a color to the solution. The color due to ferric salts may be removed by the addition of phosphoric acid, but to correct for the color due to other metals the same amount must be added to the standard. A number of metals, such as silver, lead, bismuth, and mercury, form iodates or periodates which are insoluble in dilute acids, but by using a high concentration of acid these remain in solution, since the amount of periodate added is small.

10. If a strong reducing agent, such as a ferrous salt, is present, the periodate will be reduced to free iodine which will color the solution and render it useless. All substances of this kind are removed by boiling or evaporating with nitric acid.

11. In many cases, Willard and Greathouse did not remove the color due to ferric salt, but made the proper correction by adding to

the standard the same amount of iron in the form of ferric nitrate. Their results are given in the following table:

TABLE XX  
NO PHOSPHORIC ACID ADDED; SAME AMOUNT OF IRON IN STANDARD.

Metal Number	Description of Material	Weight of Sample, Gram	KIO <sub>4</sub> Added, Grams	C Present, Per Cent	Mn Present, Per Cent	Mn Found, Per Cent	Difference, Per Cent
1	Basic Open Hearth, 1 per cent C Sample 16 . . . . .	1.0000	0.5	1.05	0.405	0.403	-0.002
2	Acid Open Hearth, 0.1 per cent C Sample 18 . . . . .	1.0000	0.3	0.10	0.412	0.409	-0.003
3	Bessemer 0.8 per cent C Sample 23 . . . . .	0.5000	0.5	0.81	0.775	0.771	-0.004
4	Acid Open Hearth, 0.2 per cent C Sample 19 . . . . .	0.5000	0.3	0.21	0.760	0.752	-0.008
5	Bessemer 0.2 C Sample 9a . . . . .	0.5000	0.3	0.25	0.918	0.913	-0.005
6	Bessemer 0.4 per cent C Sample 10a . . . . .	0.5000	0.3	0.45	0.916	0.902	-0.014
7	Iron D. Sample 6 . . . . .	0.5000	0.3	2.89	1.41	1.407	-0.003
8	Amer. Foundrymen's Assn. Iron B. . . . .	1.0000	0.5	3.11	0.415	0.404	-0.011

12. In Table XXI are recorded the results of Willard and Great-house when the same steels were used as in the preceding table but with phosphoric acid added to the sample and no iron. The phosphoric acid reacts with ferric salts to form a colorless complex ferric ion. The metals are referred to by number only.

TABLE XXI  
PHOSPHORIC ACID ADDED; NO IRON IN STANDARD.

Metal Number	Weight of Sample, Gram	KIO <sub>4</sub> Added, Gram	Mn Present, Per Cent	Mn Found, Per Cent	Difference, Per Cent
1	1.0000	0.5	0.405	0.402	-0.003
2	1.0000	0.3	0.412	0.412	±0.000
3	0.5000	0.5	0.775	0.770	-0.005
4	0.5000	0.3	0.760	0.755	-0.005
5	0.5000	0.3	0.918	0.905	-0.013
6	0.5000	0.3	0.916	0.905	-0.011

"These solutions differed only slightly in tint from the pure permanganate standard used, and such comparisons are readily made after a little practice. When more than 1 gram of iron is present its color becomes appreciable, but if the *standard* contains *no* phosphoric acid, the addition to it of 5 per cent of the weight of the iron in the sample gives sufficient color to correct for this."

13. "Two determinations were made using iron ores of known manganese content. One gram samples were dissolved in platinum dishes with 15 cc. phosphoric acid, 5 cc. hydrofluoric acid and a little nitric acid and heated until all fluoride had been volatilized. The resultant mass was dissolved in water, oxidized with 0.3 gram  $\text{KIO}_4$  and diluted to 250 cc. The usual method of dissolving the ore in hydrochloric acid could have been used equally well. To expel all chloride and to oxidize ferrous salts it is necessary to add 10 cc. of nitric acid and 15 cc. of sulfuric acid and evaporate the solution to fumes of the latter." The results are given in the following table:

TABLE XXII

Description of Ore	Weight of Sample, Grams	Mn Present, Per Cent	Mn Found, Per Cent	Difference, Per Cent
U. S. B. S. No. 28, Norrie Ore..	1.0000	0.465	0.460	-0.005
U. S. B. S. No. 29, Magnetite..	1.0000	0.07	0.068	-0.002

"In addition some twenty iron ores of known manganese content, used as 'unknowns' for students, were analyzed by the above procedure. All the common types of iron ore were represented in this series. The results obtained always agreed well with the values given by other methods." (Willard and Greathouse, *loc. cit.*)

14. Doctor Willard<sup>6</sup> has the following comments to make on the persulfate and periodate colorimetric methods for manganese: "The experience that others and myself have had with the persulfate method in the hands of students has shown that it is extremely unreliable. The periodate method, on the other hand, never goes wrong, providing the directions are followed in any reasonable manner. It will oxidize much larger amounts of manganese and the solutions thus prepared may be kept unchanged for weeks or months,—something which is

<sup>6</sup> Private communication.

hardly true even with pure permanganate. The experience of one commercial laboratory has shown the advantage of this feature. They made up a series of permanent standards and it was only necessary then in making a manganese determination to find out which standard it should be compared with,—a matter of only a minute or two. Under these conditions it becomes more rapid, I believe, than any volumetric method.

“The presence of chloride is not desirable, but if only a little is present it can be oxidized to chlorine by adding large excess of periodate. It has been stated in various references to this method that the presence of chloride would not interfere. This is true only to a limited extent and only where the operator has taken care to add sufficient periodate to oxidize all the chloride. It is preferable to remove chloride by evaporation with nitric or sulfuric acid.

“In steel analyses the use of ammonium persulfate to remove the color due to carbon is probably the most satisfactory and quickest method to accomplish this, although two other methods are given.

“If the salt  $\text{Na}_3\text{H}_2\text{IO}_6$  is used instead of  $\text{KIO}_4$  or  $\text{NaIO}_4$ , somewhat more will be required. For example, instead of 0.2 to 0.4 gram it would be necessary to use 0.3 to 0.6 gram. This other salt is cheaper to prepare and eventually the periodate will probably be marketed largely in this form. The question of obtaining a satisfactory supply of this salt at a reasonable price is one of the problems which I am now taking up in connection with some other work on periodates which I am about to publish.

“Chromium is oxidized to some extent by this reagent, the less the higher the acidity of the solution. This can be made practically negligible and compensated for by adding the same amount of chromium to the standard. With the chromium in the trivalent form the green color does not interfere seriously with the determination of manganese, but if it is oxidized to the form of chromic acid an accurate comparison becomes impossible because of the great intensity of the color.”

#### DETERMINATION OF MANGANESE IN WATER BY SODIUM BISMUTHATE <sup>7</sup>

##### Reagents.

1. Nitric acid, 4 N.
2. Sulfuric acid, sp. gr. 1.84.

<sup>7</sup> W. D. Collins and Margaret D. Foster, *Ind. Eng. Chem.*, **16**, 586 (1924).

3. Sodium bismuthate.

4. Standard permanganate solution. Dissolve 2.8769 grams of pure potassium permanganate in redistilled water, dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 1 mg. of manganese. Dilute 10 cc. of this solution to 100 cc. and thoroughly mix. One cubic centimeter of the diluted solution contains 0.1 mg. of manganese.

**Procedure.**—Measure out 100 cc. of the water or a volume small enough to contain not over 1 mg. of manganese. Place the water in a beaker, add 10 cc. of 4 N nitric acid, 1 cc. of sulfuric acid, sp. gr. 1.84, and heat on a hot-plate until most of the sulfuric acid has been driven off. Cool, and add about 50 cc. of water and 20 cc. of 4 N nitric acid which has been freed of oxides of nitrogen by bubbling air through it. Next add 0.1 gram of sodium bismuthate, stir a minute or two, allow the excess of bismuthate to settle, and filter through a Gooch crucible containing a mat of ignited asbestos which has been washed with permanganate solution. (An alundum crucible may be used in place of the Gooch.) Dilute the filtrate to a definite volume and match against a series of standards prepared by measuring out appropriate volumes of standard permanganate solution, adding to each the quantity of nitric acid added to the sample, and diluting to the same volume as that of sample.

**Notes.**

1. The sample is evaporated with nitric and sulfuric acids in order to remove chlorides and organic matter. By stopping the evaporation just before dryness the manganese sulfate is obtained in an easily soluble condition. Evaporation should be continued until almost all of the sulfuric acid has been driven off, for the reason that the shade of the permanganate is not the same in sulfuric acid and in nitric acid solutions.

2. "Practically all directions call for double treatment with bismuthate. This is necessary if the sample, when first treated with bismuthate, contains material that will be slowly oxidized by the permanganic acid after filtration from the bismuthate. If the water sample is treated as directed in the method given above, there will be no such material left and the heating to destroy the permanganic acid color is an unnecessary step which wastes time and bismuthate."

3. "Sodium bismuthate oxidizes manganese perfectly to perman-

ganic acid, so that a solution of potassium permanganate of known strength is as useful as a manganese sulfate solution for the preparation of standards. There is no advantage in reducing the manganese in a permanganate solution with oxalic acid in the presence of sulfuric acid and then using this manganese sulfate for the preparation of standards with nitric acid and bismuthate. The usual precautions in preparation and preservation of the permanganate standard solutions must be observed and a fresh standard solution should be made rather than trust one that has been kept for a long time.”<sup>8</sup>

<sup>8</sup> Collins and Foster, *loc. cit.*



## CHAPTER XXIV

### MERCURY AND MOLYBDENUM

#### DETERMINATION OF MERCURY AS SULFIDE

THIS method depends upon the formation of a colloidal suspension of mercuric sulfide and is especially adapted to the estimation of mercury in urine.<sup>1</sup>

##### Reagents.

1. Hydrochloric acid, sp. gr. 1.12 and 1.19.
2. Zinc, pure, 10 to 20 mesh.
3. Potassium chlorate. Solid.
4. Potassium acetate. Solid.
5. Hydrogen sulfide solution. Use a saturated solution, freshly prepared according to directions on page 240.
6. Standard mercuric chloride solution. Dissolve 0.1353 gram of pure mercuric chloride,  $\text{HgCl}_2$ , in water, dilute to a liter and thoroughly mix. One cubic centimeter of the solution contains 0.1 mg. of mercury.

**Procedure.**—Place 500 cc. of urine in a liter flask, add 5 grams of potassium chlorate, 50 cc. of hydrochloric acid, sp. gr. 1.12, heat until the solution turns from dark red to bright yellow. Cool to about 70° C., add 10 grams of granulated zinc, let stand 12 hours (or overnight), decant the liquid from the zinc, and wash the latter thoroughly with water. The zinc now contains the mercury. Pour 50 cc. of hydrochloric acid, sp. gr. 1.19, over the zinc, warm the mixture if necessary to make solution complete, add 20 g. of potassium acetate, transfer the solution to a 100 cc. Hohner cylinder, make up to 90 cc. with water and then to 100 cc. with a saturated solution of hydrogen sulfide. Mix thoroughly with a glass rod bent to form a ring at one end. In the meantime, a standard solution is prepared by treating 10 or 20 cc.

<sup>1</sup> A. Heinzelmann, Chem. Ztg., **35**, 721 (1911); Schumacher and Jung, Z. anal. Chem., **41**, 482 (1902).

of the standard mercuric chloride solution in the same manner as described for the sample and using the same amounts of reagents. The hydrogen sulfide should be added to the sample and standard at the same time.

#### Notes.

1. Since the mercury is in the form of a colloidal suspension of mercuric sulfide, great care must be observed to keep all conditions as to amounts of reagents, manner of mixing, etc., as near the same as possible in treating sample and standard. For the same reason, the hydrogen sulfide should be added to sample and standard at the same time and comparison of colors made at once.

2. Unless all reagents are known to be free from mercury, a "blank" must be made using the same quantities of reagents as employed in the analysis.

#### DETERMINATION OF MOLYBDENUM BY HYDROGEN PEROXIDE<sup>2</sup>

The method is based upon the brownish-red color produced by the addition of hydrogen peroxide to a faintly ammoniacal solution containing a small amount of molybdate. The results are only approximate owing to the instability of the permolybdates. The method is suitable for estimating approximately the molybdenum in rocks and ores, and in the tungsten trioxide obtained in the analysis of various minerals.

#### Reagents.

1. Nitric acid, 6 N.
2. Ammonium hydroxide, 6 N.
3. Hydrogen peroxide, 5 per cent.
4. Standard molybdate solution. Dissolve 0.4292 gram of pure ammonium molybdate of commerce,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , in water, dilute to a liter, and mix thoroughly. One cubic centimeter of the solution contains 0.05 mg. of  $\text{MoO}_3$ .

**Procedure.**—The solution of sample is evaporated almost to dryness and tested with litmus. If alkaline, neutralize with nitric or sulfuric acid, make faintly ammoniacal, add 3 drops of a 5 per cent hydrogen peroxide solution, and compare at once, without dilution, with a freshly prepared standard.

<sup>2</sup> W. Bettel, Chem. News, **79**, 40 (1908).

**Notes.**

1. The reddish-brown color is partly discharged by dilution, and disappears on standing, oxygen being evolved. The color is instantly discharged by the addition of excess alkalis. A moderate excess of ammonia has very little action on the color but a large excess discharges it.

2. As little as 0.001 mg. of  $\text{MoO}_3$  in several cubic centimeters of solution can be detected by a very faint tint. 0.005 mg. of  $\text{MoO}_3$  in the same volume gives a distinct reaction.

**DETERMINATION OF MOLYBDENUM BY TANNIC ACID<sup>3</sup>**

When a small volume of a dilute tannic solution is added to an acetic acid solution containing a little molybdate a color is obtained, the intensity of which is proportional to the concentration of molybdenum. The method is recommended for the determination of molybdenum in materials containing 2 per cent or less of the metal.

**Reagents.**

1. Hydrochloric acid, 6 N.
2. Nitric acid, 6 N.
3. Acetic acid, 99.5 per cent.
4. Tannic acid, 0.5 per cent solution freshly prepared.
5. Ammonium hydroxide, 6 N.
6. Standard ammonium molybdate solution. Dissolve 9 grams of pure ammonium molybdate of commerce,  $(\text{NH}_4)_6 \text{Mo}_7 \text{O}_{24} \cdot 4\text{H}_2\text{O}$ , in water, dilute to a liter and thoroughly mix. Standardize against pure lead sulfate. (0.3 gram  $\text{PbSO}_4 = 0.1584$  gram  $\text{MoS}_2$ .)

**Procedure.**—One or 2 grams of the sample are treated in a casserole with 10 cc. of nitric acid, heated gently for 30 minutes, and then evaporated just to dryness. Add 30 cc. of water, 10 cc. of hydrochloric acid, warm, stir, add 15 cc. of ammonium hydroxide, boil for several minutes, filter into a 250 cc. volumetric flask and wash the precipitate three or four times with small quantities of hot water. Wash the precipitate back into the casserole, dissolve in 10 cc. of hydrochloric acid and reprecipitate with 15 cc. of ammonium hydroxide, filter into the flask containing the first filtrate, wash, and make just acid with acetic.

<sup>3</sup> G. Spurge, Chem. Eng. Mining Rev., 11, 258 (1919).

Then add 10 cc. of concentrated acetic acid, cool, dilute to the mark, and mix.

Next prepare a standard by transferring 2 cc. of the ammonium molybdate solution to a 250 cc. volumetric flask, adding 20 cc. of hydrochloric acid, 30 cc. of ammonium hydroxide, boiling a few minutes, making just acid with acetic acid, adding 10 cc. of concentrated acetic acid, cooling, diluting to the mark, and mixing.

The comparison is made as follows: Place 2 cc. of fresh tannic acid solution in a 50 cc. Nessler cylinder, add 2 cc. of the standard, dilute to the mark and mix. Then place 2 cc. of the tannic acid solution in another 50 cc. Nessler cylinder and run in the solution of sample until the colors match, after finally diluting to the mark and mixing.

**Note.**—If tungsten is present, filter off the first hydrochloric acid solution before adding ammonium hydroxide.

#### DETERMINATION OF MOLYBDENUM IN TUNGSTEN

##### METHOD OF KING<sup>4</sup>

This method is a modification and refinement of the method of L. Leley, Philips' Lamp Works, Holland, and is based upon the formation of a blood-red compound of molybdenum thiocyanate when a thiocyanate salt is added to a solution of reduced molybdenum. Ether is used as a solvent for extracting the molybdenum thiocyanate and the colored solution is matched in a modified Campbell-Hurley colorimeter. The method is especially adapted for the determination of quantities of molybdenum in tungsten less than 300 p.p.m. and gives trustworthy results when the molybdenum content is as low as 10 p.p.m.

##### Reagents.

1. Nitric acid, sp. gr. 1.42 and 6 N.
2. Hydrochloric acid, 2 N and 1 N (approx. 1 : 5 and 1 : 10, respectively).
3. Hydrofluoric acid.
4. Tartaric acid. Dissolve 600 grams of C. P. tartaric acid in 700 cc. of boiling water, cool, and make up to a liter.
5. Sodium hydroxide, 0.5 N. Dissolve 20 grams of C. P. sodium

<sup>4</sup> Ind. Eng. Chem., **15**, 350 (1923).

hydroxide (electrolytic) in 600 cc. of distilled water, dilute to a liter, allow to stand until the ferric hydroxide precipitate settles, and then carefully decant (or siphon) into a clean bottle.

6. Potassium thiocyanate, 30 per cent.

7. Stannous chloride. Dissolve 150 grams of stannous chloride;  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , in 100 cc. of concentrated hydrochloric acid, boil until clear, and add 250 cc. of distilled water. Place a few pieces of metallic tin in the solution to prevent oxidation.

8. Ether. The ether used for the extraction should be tested with potassium iodide and starch. If a blue color develops, the ether should be shaken with a 10 per cent solution of sodium thiosulfate and redistilled. A blank test should give no color after several hours' standing. The precaution of redistilling has never been found necessary.

9. Standard sodium molybdate solution. Pure molybdic trioxide is prepared by igniting C. P.  $(\text{NH}_4)_2\text{MoO}_4$  to  $\text{MoO}_3$  and resubliming in silica or platinum dishes. The sublimed oxide is dissolved in a small excess of a 10 per cent NaOH solution, neutralized with dilute HCl and then made alkaline to litmus. The solution above is diluted to approximately 0.1 N, after which the molybdenum is accurately determined by precipitating and weighing as  $\text{PbMoO}_4$ . A portion of this solution is diluted so that 1 cc. will contain 0.05 mg. molybdenum.

The following description of apparatus, Fig. 50, and procedure have been taken from the work of King.<sup>5</sup>

**Apparatus.—Colorimeter.**—The colorimeter is shown in Fig. 50. The colorimeter tubes with attached reservoir are made of Pyrex glass. They have an approximate capacity of 250 cc., an inside diameter of 4 cm., and an approximate height of 23 cm. The bottoms of the tubes are clear glass made by sealing pieces of large flasks on the bottoms, which have been previously ground to a plane. The tops of the tubes are ground level so that the plate-glass covers fit snugly over the tops. The tube for the standard is calibrated to contain 250 cc. and marked on the side-arm. A separate scale equal to the length of the side-arm to mark, and graduated in 100 equal divisions, is fastened behind the side-arm. The use of a separate scale with a white background and black markings facilitates rapid and accurate readings. The length of the side-arm of the sample tube is equal to the length of side-arm of the standard tube. The side-arm is

<sup>5</sup> *Loc. cit.*

divided into 10 equal divisions and etched on the glass. Fractional readings of the large divisions are made with a movable glass sleeve fitted with a paper scale equal to the length of a large division

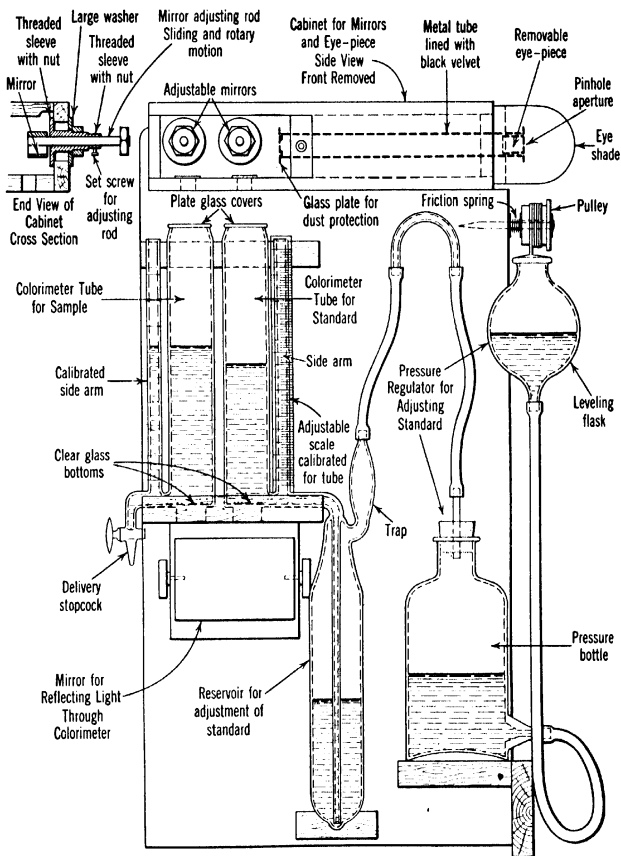


FIG. 50.—King Colorimeter for Molybdenum.

and subdivided into 10 equal divisions. This device saves extra calibrating and provides accurate readings from 1 to 100. No correction for the taper of the tube is necessary if the diameters of the tubes

are reasonably uniform and the inside diameters of their opposite ends do not vary more than 0.4 mm. The correction for the difference in the volumes of the tubes is equal to volume of sample tube (to mark) divided by volume of standard tube (to mark). The volume of the reservoir (to mark) is equal to the volume of standard tube and side-arm to calibrated mark. The trap prevents the loss of ether solution while mixing the standard.

The cabinet is a dust-proof box inclosing two mirrors and telescope. The mirrors are supported with a mechanical device to give three adjustments: (1) rotary movement, (2) sliding movement parallel to axis, (3) displacement movement relative to the position of the telescope and the direction of the light from the tubes. Glass plates covering the openings in the bottom of the box transmit light from the sample and standard tubes to the mirrors. The observation tube is 20 cm. long, 1.2 cm. in diameter, and is lined with black velvet to prevent disturbing reflections. The pin hole aperture in the removable eyepiece is 1 mm. in diameter, while the opening in the opposite end of the telescope is 4 mm. An eyeshade is attached to the end of the telescope, which cuts off light from the outside and permits the observer to keep the left eye open while making observations.

The height of the standard solution is controlled by a pressure regulator. The pressure bottle has a capacity of 500 cc. and is half filled with water saturated with ether. By turning the pulley the height of the leveling flask regulates the amount of water and air in the pressure bottle, which automatically provides pressure regulation for the standard tube. The purpose of introducing a pressure bottle is to prevent the evaporation of ether from the adjustment reservoir. The pulley has a friction spring which fixes the height of liquid in leveling the flask at any desired level.

The mirror for reflection is swung in position to reflect the light from the white plate-glass reflector in the rear of the colorimeter up through the colorimeter tubes into the openings at the bottom of cabinet holding the adjustable mirrors.

*Shaking Flask and Siphon.*—The shaking flask consists of a 300 cc. flask sealed to a 400 cc. short-neck (3 cm.) flask and shaped at the mouth to fit a small rubber stopper. A small opening is made near the mouth to admit air. After shaking out a solution the ethereal layer is removed by properly adjusting a 3 mm. capillary siphon, inserting

the rubber stopper, and forcing air through the opening until all the ether is forced out.

*Illuminating Equipment.*—A 500-watt "Trutint," north skylight unit provides the source of light both for the extraction process and color matching. This type of lighting unit is equipped with a blue-glass color filter and is designed to give a quality of light closely approaching north skylight. A dependable source of light, reasonably uniform both in quality and quantity and reproducible at will, makes possible accurate color observations at all times, a condition which is not possible with daylight. To obtain the best conditions of light diffusion, the glass filter is sand-blasted and the diffused light reflected directly into the colorimeter by a polished plate of white glass.

*Procedure.—Solution of Sample.*—Tungsten and molybdenum must be in the form of their alkali salts. Tungsten metal is oxidized in the wet way. Weigh 4 grams of metal into a 200 cc. platinum dish, cover with 30 to 40 cc. of hydrofluoric acid, and add, drop by drop, concentrated or dilute nitric acid, as the violence of the reaction indicates, until the tungsten is dissolved. Evaporate the solution nearly to dryness, add concentrated nitric acid, and evaporate to dryness. Repeat acid treatment three times, evaporating to dryness each time. Ignite at a low temperature not to exceed 550° C. Ignition of all tungsten samples is necessary to eliminate organic matter and to break up the tungstic acid hydrates and complexes.

Tungstic acid is treated three times with concentrated nitric acid, evaporated to dryness, and ignited. Tungstates soluble in caustic are treated directly. Boil a 5 gram sample, after treatment as directed above, with 25 cc. sodium hydroxide solution (35 cc. for sample over 12 grams) until solution is complete. To the solution add 75 cc. hot water and boil. No appreciable residue of tungsten should remain. Fusion of sample of tungstic oxide with sodium and potassium carbonates is apt to cause a slight loss of molybdenum. Filter the solution in a Gooch crucible on an asbestos mat, wash with 5 per cent sodium chloride solution, and transfer to a 250 cc. calibrated flask and dilute to mark.

*Preliminary Treatment.*—Transfer a 75 cc. aliquot to a 500 cc. Erlenmeyer flask, and neutralize with dilute hydrochloric acid (1 : 10), using neutral litmus paper. At no time should a precipitate of tung-



stic acid be allowed to form. When the solution is slightly acid, immediately add tartaric acid solution proportionate to the amount of tungsten present, using 20 cc. of solution for every 6 grams of  $WO_3$  or fraction. Transfer to 400 cc. shaking flask, add 25 cc. dilute hydrochloric acid (1 : 5) and 5 cc. of a 30 per cent potassium sulfocyanate solution, and dilute to approximately 350 cc. Cool to 5° or 10° C.

*Extraction with Ether.*—To the cooled solution add 20 cc. of stannous chloride solution. Thorough mixing is best done by blowing forcibly into the pipette and directing the emerging stream into the solution. Immediately add 90 cc. ether and sufficient water to bring the water meniscus up to the bottom of the constriction when the two layers are saturated with each other. Insert a cork in the flask, hold the finger over the small opening at the top, and shake the flask vigorously one-half minute. Cool the flask in ice water until the ether layer separates. Adjust the siphon and transfer the ether to the colorimeter tube (left). Add a 50 cc. portion of ether to the shaking flask, and repeat the extraction as long as any color is removed. (See Note 1.)

*Standard Ether Solution.*—Standard ether solution is prepared by adding molybdenum to specially purified tungsten and shaking out in the regular way. A suitable quality of purified tungsten very low in molybdenum may be obtained by recovering and purifying the tungstic acid from the aqueous solutions of the ether extraction. Purification is best made by ammonium paratungstate precipitation followed by nitric acid digestion. Weigh 3 grams of ignited oxide into a 500 cc. flask, add 10 cc. of a standard molybdenum solution, equivalent to 0.5 mg. molybdenum, and follow the procedure to the extraction with ether. After shaking the solution out with ether, transfer the ether solution to the colorimeter tube (right) and continue extracting with 50 cc. portions until the reservoir is filled to mark. Mix the standard by blowing air through the reservoir, replacing the evaporated ether if necessary, using only the ether which has been shaken out. The volume of the reservoir filled to the mark is equal to 100 scale divisions. Loss of ether is largely prevented by covering the tops of the color tubes.

*Matching of Colors.*—The standard solution in the right tube is matched against the unknown solution in the left tube. To accomplish this, disconnect the rubber tubing from the trap, fill the reservoir to the mark, and lower the leveling flask until the pressure bottle is empty. Connect the rubber tubing again to the trap. With the light

properly adjusted behind the colorimeter, the reflecting mirror is turned so that uniform illumination is provided in both tubes. Manipulate pulley so that the height of the solution in the right tube is regulated to give equal color densities in both tubes, and note reading on the scale. With a little practice readings can be duplicated within one or two scale divisions. If the color reading is over 90 divisions, fill the left tube to a convenient mark, mix and drain off sufficient ether to permit the readings to fall between 70 and 90 scale divisions. The volume of the ether aliquot may be accurately measured by using the calibrated sleeve described under "Colorimeter." When it is necessary to remove more than half of the ether aliquot, a second but smaller sample is taken and sufficient purified tungstic acid, from which the standard was prepared, added to make a total of 1.5 to 3 grams of  $\text{WO}_3$ . If the color reading is less than 50 divisions a proportionally larger sample is taken so that an approximate reading of 75 divisions is obtained. However, the quantity of tungstic acid used in making the standard need not be increased except where great refinement is desired.

The following notes are from the work of King:<sup>6</sup>

#### Notes.

1. When aqueous solutions turn slightly blue after the addition of stannous chloride some of the molybdenum stays in a form which cannot be shaken out. In some cases over 20 per cent of the molybdenum remains undetected. This is probably due to the formation of hydrated or complex compounds of tungsten when precipitated metatungstic acid or insoluble residues are redissolved. The resolution of the fine precipitate by the addition of tartaric acid is not sufficient to prevent the formation of the blue color. In several cases a poor quality of tartaric acid has been known to cause a blue color, but this can be detected by a blank test. When an appreciable quantity of blue color has formed it has been considered a safer plan to discard the determination and start a new one.

2. In preparing a reliable procedure for colorimetric analysis of molybdenum, the importance of adopting uniform conditions cannot be too strongly emphasized. This applies especially to the quality of reagents and the conditions affecting their concentration. New reagents should be tested by running blank determinations. After the addition of the sulfocyanate an analysis should be carried through

<sup>6</sup> *Loc. cit.*

promptly.\* Turbidity or precipitation occurring in either solution prevents the complete extraction of molybdenum, or makes accurate color matching impossible. Although standard solutions have stood over a week without measurable deterioration, it is probably better to prepare a fresh solution each day and check against a standard sample of tungstic acid. The stability of the solutions is probably due to the reducing action of stannous chloride held in solution by ether.

3. None of the elements commonly associated with tungsten ores interferes with the molybdenum determination. The following elements do not interfere: iron, copper, manganese, nickel, cobalt, titanium, columbium, tantalum, uranium, chromium, vanadium, thorium, aluminum, phosphorus, calcium, arsenic, and silicon.

4. The colorimetric analysis of samples of  $\text{WO}_3$  containing 321, 151, 48, and 29 p.p.m. molybdenum, respectively, showed a variation between the maximum and minimum value for each sample of less than 10 per cent of the total molybdenum present.

5. The size of the sample should be adjusted so that a scale reading of 60 to 90 divisions is obtained, thus reducing to a minimum the error of color matching.

#### DETERMINATION OF MOLYBDENUM IN STEEL<sup>7</sup>

This is a rapid method for the estimation of molybdenum in steel and is based upon a combination of the colorimetric method of the U. S. Steel Corporation and that of King (see page 285)<sup>8</sup>. It has been used in the determination of the molybdenum content in the charges of electric furnaces, the molybdenum content ranging from 0.02 to 0.15 per cent. Only 20 to 25 minutes are required to make a determination.

#### Reagents.

1. Nitric-sulfuric acid mixture. Add 350 cc. of nitric acid, sp. gr. 1.42, and 225 cc. of sulfuric acid, sp. gr. 1.84, to 750 cc. of water.

2. Hydrochloric-sulfuric acid mixture. Add 450 cc. of sulfuric acid, sp. gr. 1.84, and 100 cc. of hydrochloric acid, sp. gr. 1.19, to 1450 cc. of water.

3. Potassium thiocyanate, 5 per cent. Dissolve 50 grams of potassium thiocyanate in a liter of water.

4. Stannous chloride. Dissolve 250 grams of stannous chloride,  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , in 200 cc. of hydrochloric acid, sp. gr. 1.19, and boil until

<sup>7</sup> O. L. Maag and C. H. McCollam, *Ind. Eng. Chem.*, **17**, 524 (1925).

<sup>8</sup> *Methods of the Chemists of the U. S. Steel Corporation for the Sampling and Analysis of Alloy Steels*, 2d ed., p. 72, 1921.

clear. Dilute with 800 cc. of water and add a few pieces of metallic tin to prevent oxidation.

5. Ether. (See p. 286.)

**Procedure.**—Place a 0.5 gram sample of the steel in a 250 cc. beaker, add 10 cc. of the nitric-sulfuric acid mixture, heat gently until solution is complete, and then evaporate carefully and rapidly on a hot-plate until copious fumes are evolved. Do not use a cover glass. A little practice may be required in regulating the heating so as to avoid spattering. All nitrates must be driven off, otherwise concordant results will not be obtained.

After heating till all nitrates have been driven off, cool the contents of the beaker, add exactly 30 cc. of the hydrochloric-sulfuric acid mixture and boil until the salts have dissolved. (See Note 1.) Cool the solution to room temperature; add from a burette 5 cc. of 5 per cent potassium thiocyanate solution, and stir thoroughly; add from a burette 10 cc. of the stannous chloride solution, and again stir thoroughly. (See Note 2.) Transfer the acid solution to a separatory funnel, add 10 cc. of ether and shake thoroughly. Return the acid solution to the original beaker and run the ether solution into a clean, dry, 50 cc. graduated cylinder. Repeat shaking the acid solution with successive 10 cc. portions of ether until all color has been removed. Transfer the combined ether solutions to a graduated colorimeter tube (see Note 3), place the tube in the colorimeter (Kennicott-Campbell-Hurley is a good type for this determination) and match against a standard solution prepared as follows: Weigh out two 0.5 gram samples of standard steel and treat them as directed above for the sample. Make the ether extracts up to exactly 50 cc. in the standard matching tubes, place in the colorimeter and compare. If they have the same color tint, one of them may be diluted to exactly 100 cc. as a standard for low percentage molybdenum, or the two may be united to make a standard for steels with a higher molybdenum content. Transfer the standard solution to the leveling tube of the colorimeter and match the color of the sample solution. From the colorimeter reading (average of several) calculate the per cent of molybdenum in the sample.

#### Notes.

1. It is important to use the proper amount of hydrochloric acid in dissolving the salts. "Too much will cause a fading of the color

even before the ether extraction can be made, while not enough of this reagent may present difficulties in the solution of the salts.”<sup>9</sup>

2. All of the iron must be reduced before the ether extraction is made; therefore, after the stannous chloride is added care should be taken that no unreduced solution is left on the sides of the beaker.

3. Approximately the same volume of ether should be used in extracting the sample and standard steels.

4. “The top of the leveling tube should be covered when not in use, in order to increase the time that a standard can be used. Since the deterioration of a standard is generally due to evaporation of the ether rather than breaking down of the salt, its life is largely dependent on the room temperature. In this laboratory the standard is renewed every 2 hours, two samples of standard steel being kept fuming slightly on the cooler portion of the hot-plate, to be used whenever the standard in use is suspected.”<sup>9</sup>

<sup>9</sup> Maag and McCollam, *loc. cit.*

## CHAPTER XXV

### NICKEL

#### DETERMINATION OF NICKEL AS THE DIMETHYLGLYOXIME

THIS method is based upon the formation of nickel dimethylglyoxime,  $\text{Ni}(\text{C}_4\text{H}_7\text{N}_2\text{O}_2)_2$ , a scarlet-red compound. The reaction is carried out in a faintly ammoniacal solution. Since nickel dimethylglyoxime is very slightly soluble, only minute amounts of nickel can be tested, for otherwise a precipitate settles out.

#### Reagents.

1. Hydrochloric acid, 6 N.
2. Nitric acid, sp. gr. 1.42.
3. Ammonium hydroxide, 6 N.
4. Sodium hydroxide, 6 N.
5. Hydrogen sulfide. See p. 240.
6. Dimethylglyoxime, 0.1 N. Dissolve 12 grams of the solid in 1000 cc. of 95 per cent alcohol.

**Procedure.**—Dissolve the sample, add 2 or 3 cc. of concentrated nitric acid and boil a few minutes. Any iron or phosphate present is then precipitated with an excess of ammonium hydroxide. If a precipitate forms, filter, dissolve the precipitate in dilute hydrochloric acid, reprecipitate with ammonium hydroxide, filter, and wash. If much iron is present another precipitation should be made. Finally, combine the filtrates and evaporate to dryness, dissolve the residue in 5 cc. of 6 N hydrochloric acid, dilute to about 100 cc., heat to boiling, and pass hydrogen sulfide into the hot solution. Filter, boil off the hydrogen sulfide in the filtrate, add sodium hydroxide to precipitate the nickel, filter, convert the nickel hydroxide into nickel chloride by adding a slight excess of hydrochloric acid, make up to a definite volume, and mix thoroughly. To the solution, or an aliquot part, add ammonium hydroxide in slight excess and then 2 cc. of dimethylglyoxime. Mix and compare the resulting color with that of a standard nickel solution prepared at the same time.

**Notes.**

1. One part of nickel in 400,000 parts of solution can be detected.<sup>1</sup>
2. A tenth of a milligram of nickel in the presence of 500 mg. of cobalt can be distinctly recognized. However, when cobalt is present, a large excess of ammonia is added to the solution before adding the dimethylglyoxime.
3. When cobalt is present, dimethylglyoxime equivalent to the cobalt must be added before a small quantity of nickel will react. This is probably due to the cobalt combining with the reagent to form a soluble complex salt.

**DETERMINATION OF NICKEL BY POTASSIUM THIOCARBONATE**

The method is based on the rose-red to brown-red color developed with potassium thiocarbonate and ammoniacal nickel solutions.<sup>2</sup> The nickel may be present as sulfate, nitrate, or chloride before addition of ammonium hydroxide.

**Reagents.**

1. Nitric acid, sp. gr. 1.42.
2. Ammonium hydroxide, 6 N.
3. Potassium thiocarbonate. Dissolve 4 grams of potassium thiocarbonate,  $K_2CS_3$ , in water and dilute to 100 cc.
4. Standard nickel solution. Dissolve 0.6729 gram of nickel ammonium sulfate,  $NiSO_4 \cdot (NH_4)_2 SO_4 \cdot 6H_2O$ , in water, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 0.1 mg. of nickel.

**Procedure.**—A sample containing between 0.34 and 2.0 mg. is weighed out and dissolved in hydrochloric, sulfuric, or nitric acid, the latter usually, being the quickest. If the sample is a water-soluble salt, the solution is made slightly acid. Add ammonium hydroxide to the solution until it is almost neutral, then 0.5 cc. of the potassium thiocarbonate solution, finally adding ammonium hydroxide in excess and diluting to 20 cc. Compare at once with a standard that has been prepared along with the sample, using the same volume (20 cc.) and similar Nessler tubes.

<sup>1</sup> L. Tschugaeff, Ber., **38**, 2520 (1905).

<sup>2</sup> V. Lindt, Z. anal. Chem., **53**, 165 (1914).

**Notes.**

1. The volumes of the sample solution and of the comparison solution must be the same (20 cc.) and must be compared in similar tubes.

2. The solutions, both sample and comparison, must be mixed with the reagent and compared at the same time; solutions differing in age cannot be used.

3. The potassium thiocarbonate solution must be prepared fresh once a week.

4. Metals of the hydrogen sulfide group must be removed. This is accomplished by precipitation with hydrogen sulfide in an approximately 0.3 N acid solution. Manganese and iron must also be absent. These are removed by boiling with bromine water or hydrogen peroxide (after removal of  $H_2S$ ) and then adding ammonium hydroxide in slight excess. Cobaltous compounds interfere, but the bromine or hydrogen peroxide treatment oxidizes them to cobaltic compounds which are non-interfering. Zinc slowly forms a finely divided white precipitate but this does not interfere with rapid work unless a considerable amount is present. In case of doubt, add a similar quantity of zinc to the standard.

5. Analyses of nickel solutions, Ni steel and other alloys showed excellent agreement with the dicyanodiamidine and electrolytic methods. Representative experimental results obtained by Lindt<sup>3</sup> are given in the following tables:

TABLE XXIII  
Ni STEEL

Colorimetric Per Cent Ni	Mean	Dicyano- diamidine Per Cent Ni	Difference
4.73	4.83	4.82	+0.01
4.92			
4.86			
2.97	2.95	2.96	-0.01
2.93			
2.92			
2.99			

<sup>3</sup> *Loc. cit.*



TABLE XXIV

## BRASS

Composition: Cu, 61.552 per cent; Pb, 0.164 per cent; Fe, 0.306 per cent; Ni, 13.668 per cent; Zn, 24.310 per cent. Cu, Pb and Fe removed but not the Zn.

Colorimetric Per Cent Ni	Electrolytic Per Cent Ni	Difference, Per Cent	Mean, Per Cent
13.63	13.668	-0.038	-0.015
13.63	13.668	-0.038	
13.70	13.668	+0.032	

## DETERMINATION OF NICKEL BY POTASSIUM DI-THIO-OXALATE

METHOD OF FAIRHALL <sup>4</sup>

This method is based upon the deep magenta color obtained when potassium di-thio-oxalate is added to solutions containing minute amounts of nickel. The reaction was first observed by Jones and Tasker.<sup>5</sup> It not only is more sensitive than any other colorimetric method for nickel analysis, but also permits the accurate determination of small fractions of a milligram.

The method described below was developed for the determination of nickel in the minute quantities that occur in biological material.

**Reagents.**

1. Hydrochloric acid, sp. gr. 1.19, and 1 : 1.
2. Acetic acid, glacial.
3. Ammonium hydroxide. Prepare by absorbing ammonia gas in cold distilled water.
4. Ammonium acetate, 50 per cent.
5. Ammonium oxalate, 0.5 N. Dissolve 40 grams of ammonium oxalate,  $(\text{NH}_4)_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ , in water and dilute to a liter.
6. Sodium citrate, 20 per cent.
7. Bromine.
8. Hydrogen sulfide. See p. 240.
9. Potassium di-thio-oxalate.<sup>6</sup> The following method of prep-

<sup>4</sup> L. T. Fairhall, J. Ind. Hyg., 8, 528 (1926).

<sup>5</sup> J. Chem. Soc., 96, ii, 1904 (1909).

<sup>6</sup> This salt may be obtained from Eastman Kodak Co., Rochester, N. Y.

aration of potassium di-thio-oxalate is that of Jones and Tasker<sup>7</sup> as modified by Fairhall. (See Note 1.) Treat 2 gram-molecules of ethyl mercaptan with 1 gram-molecule of oxalyl chloride at room temperature, adding the oxalyl chloride in small amounts at a time. Hydrogen chloride is abundantly evolved. After action has apparently ceased, heat gently in order to drive off the remaining excess of hydrogen chloride and an excess of either of the reacting substances. Dissolve the resulting ethyl di-thio-oxalate in ethyl alcohol and add an equivalent of potassium hydrosulfide dissolved in alcohol. The latter should be prepared by treating an equivalent weight of potassium hydroxide in alcohol with hydrogen sulfide until the cold solution is saturated with hydrogen sulfide gas; it should be allowed to stand overnight and then be filtered from the residue of metallic sulfides. Pour the alcoholic potassium hydrosulfide solution into the solution of ethyl di-thio-oxalate, stirring vigorously, and allow to stand for about an hour. An abundant white crystalline precipitate of potassium di-thio-oxalate forms. Pour off the supernatant fluid, collect the crystals on a Büchner funnel, and wash well with cold alcohol. The crystals should be dried and stored in amber-colored bottles. The salt will keep for several months but eventually becomes light coffee-colored and gives the characteristic magenta color with nickel accompanied by a turbidity in acid solution which interferes with accurate readings.

#### Procedure A: Preliminary Treatment

(1) *Drying and Carbonizing*.—The material (tissues, excreta, foodstuffs, milk, etc.), must be dried, baked in an oven until charred, and then ashed, preferably in an electric muffle furnace. Transfer the material to a porcelain dish and heat to dryness on a hot-plate or in an oven. When dry, increase the temperature so that the material is carbonized. Transfer the char to a small porcelain dish for ashing. Urine or milk should be boiled to dryness in a large porcelain dish on a hot-plate. Vigorous boiling is to be avoided, and, in order to prevent bumping and spattering, add 10 cc. of concentrated hydrochloric acid. Char the residue as above and transfer to a small porcelain dish.

(2) *Ashing*.—Transfer the dishes of carbonized material to the muffle furnace and heat at a low red heat. They should remain at this temperature no longer than half an hour as the salts (in the case of urine and certain foodstuffs) fuse and coat the carbon particles, pre-

<sup>7</sup> *Loc. cit.*

venting any further oxidation and making the subsequent extraction difficult. It is better to underheat the first time than to overheat. After the dishes have been removed and are cold, treat them with 15 cc. of 1 : 1 hydrochloric acid, cover the residue with water, and heat to boiling, first covering the porcelain dishes with clock glasses. Filter and extract twice more with boiling water. Return the filter paper and residue to the porcelain dish, dry in a hot-air oven, and re-ash in the muffle furnace. When cold, extract as before. One more ignition and extraction will generally be necessary before everything goes into solution. There is usually a siliceous residue from feces, which may be discarded.

(3) *Removal of Heavy Metals.*—Neutralize the hydrochloric acid solution with pure ammonium hydroxide, using methyl orange as an indicator. Then add a few drops of hydrochloric acid until the solution is just acid in reaction. Saturate the cold solution with hydrogen sulfide gas and allow it to stand a few hours or overnight if no precipitate forms at once. Filter and wash the residue with hydrogen sulfide water. Boil the filtrate till free from hydrogen sulfide and add bromine water in sufficient amount to oxidize the iron to the ferric state. The solution is then analyzed for nickel by one of the two following methods.

#### **Procedure B: Determination of Nickel**

(1) *In the Absence of Cobalt.*—It is necessary to free the solution from iron only in order to estimate the nickel directly. Most biologic material contains iron in varying amounts, accompanied usually by a greater amount of phosphate. Ferric phosphate is insoluble in neutral or acetic acid solution, and the iron may be removed, therefore, by merely neutralizing the solution. This must be done carefully, however, in order to avoid precipitating calcium and magnesium phosphates. The best plan is to add a buffer salt such as ammonium acetate. The slightly acid solution containing ferric iron should be treated with 10 cc. of 50 per cent ammonium acetate solution and 0.5 cc. of glacial acetic acid. All the free hydrochloric acid is neutralized by the ammonium acetate, and an equivalent amount of acetic acid is liberated. The acetic acid prevents the precipitation of calcium and magnesium phosphates. The solution *must be cold* before the ammonium acetate is added, otherwise part of the iron will be reduced and thus escape precipitation. Filter the cold solution through quanti-

tative filter paper into a volumetric flask. The filtrate should be water-white with no trace of turbidity. The ferric phosphate is usually small in amount and is readily filtered out. Dilute the solution to an exactly known volume, say 500 cc.

*Nickel Reading.*—Transfer 50 cc. of this solution to a Nessler tube and add a small amount of potassium di-thio-oxalate. If nickel is present, a clear magenta color develops at once. If nickel is absent, no color is apparent except that the solution may be slightly yellowish from an excess of di-thio-oxalate. A brownish color indicates an excess of cobalt in solution; a purple shade shows that the iron has not been completely removed, and in this event it will be necessary to repeat the oxidation and precipitation with an aliquot part of the solution. This is very troublesome and it is far easier to insure complete oxidation of the iron at the earlier stage.

In order to determine the amount of nickel present, a series of nickel standards in Nessler tubes should be prepared, ranging from 0.005 to 0.05 mg. of nickel—i.e., 0.005, 0.01, 0.02, 0.03, 0.04, 0.05 mg. The unknown solution should be matched against these. It is not advisable to use higher concentrations of the standard nickel solution with Nessler tubes, as the tint is too deep for satisfactory reading. For larger amounts of nickel, it is preferable to use a colorimeter of the Duboscq type.

(2) *In the Presence of Cobalt.*—In case cobalt is present in amount sufficient to interfere with the colorimetric test for nickel, separation of the nickel from the cobalt will be necessary before the test is applied. Iron, if present in the solution, may be prevented from precipitating at a later stage (as the hydroxide) by the addition at this point of sodium citrate. Add 10 cc. of 20 per cent sodium citrate solution. Then add ammonium oxalate solution very slowly, stirring constantly, so as to separate calcium oxalate in crystalline form in the acid solution. When precipitation ceases, add dilute ammonium hydroxide slowly in such a manner as to produce crystalline ammonium magnesium phosphate in the same solution without filtering out the calcium oxalate. After the calcium and magnesium are quantitatively precipitated, they should be filtered out, washed, redissolved in hydrochloric acid, and reprecipitated as before. The filtrate from this precipitation should be combined with the first filtrate. In this way any nickel that is mechanically carried down with or incompletely washed out of the first precipitate may be recovered. The alkaline filtrate, which is

now free from calcium and magnesium, and partly free from phosphates (the latter are removed only as a means of eliminating magnesium), is treated with an excess of  $\alpha$ -benzyl-dioxime, filtered, the residue dissolved in acid—aqua regia is preferable—and the acid solution evaporated to dryness in a porcelain dish. Add 1 or 2 cc. of concentrated hydrochloric acid and again evaporate to dryness in order to destroy any excess of nitric acid. Finally, dissolve the residue in a few drops of dilute hydrochloric acid, dilute to a convenient known volume, and determine the nickel content by the colorimetric method already outlined. (See Note 2.)

#### Notes.

1. It has been found that different lots of potassium di-thio-oxalate vary somewhat in the rate at which the color of the compound changes in acid solution with amounts of nickel of less than 0.05 mg. The rate of change is not significant in solutions of low acidity, but in strongly acid solutions the color change is noticeable after an hour or more and the Nessler tube readings are affected by a turbidity which develops. Apparently the method of preparation is partly at least responsible for this, as one lot (used by Fairhall) in particular was but little affected by changes in acidity.

2. The second method is longer and more troublesome than the first, and fortunately need be utilized only in those cases where a noticeable amount of cobalt is present. The small amount of cobalt existing in nickel or nickel salts is insufficient to have any effect on the color which develops and, judging from the analyses obtained so far, it is only occasionally that cobalt is excreted in sufficient amount to interfere with the test.

3. An examination of this colorimetric method has shown that nickel in amounts of 1 mg. can be determined with an average accuracy of 0.02 mg. To test this, a solution was made up of pure salts in imitation of the mineral content of milk. Exactly 1 mg. of nickel was added to portions of this salt solution, each of which corresponded in mineral content to 500 cc. of milk. These solutions were then analyzed for nickel by the procedure outlined above. In a series of ten experiments the maximum deviation was 0.05 mg. and the average error 0.02 mg.

Experiments in which the acidity of the solution was varied, using hydrochloric acid in one case and acetic acid in the other, showed that

the determination of nickel in amounts of 0.1 mg. or more is not seriously affected by varying the acid concentration from 0.0001 molar to 1.0 molar. Estimations made of the nickel content of solutions of nickel in acid solutions of various strengths are summarized in Table XXV. The depth of color of these solutions when treated with potassium di-thio-oxalate was compared with a standard of the same concentration in distilled water, using a Duboscq colorimeter for comparison. It is apparent from these results that readings of this amount of nickel can be made with accuracy in solutions ranging from 0.0001 to 1.0 molar in acidity.

TABLE XXV  
EFFECT OF ACID CONCENTRATION ON NICKEL ESTIMATION

Amount of Nickel, Mg	Acid Concentration (Molar)	Hydrochloric Acid	Acetic Acid
0.1	1.0	0.098	0.097
0.1	0.1	0.099	0.101
0.1	0.01	0.094	0.101
0.1	0.001	0.098	0.098
0.1	0.0001	0.098	0.097

In the method of analysis as above outlined, the degree of acidity of the final solution which is used for the nickel reading will be no more than 0.02 molar. When the amounts of nickel are less than 0.05 mg., the acidity should be adjusted, however, so that the solution is no more than 0.01 molar with respect to acid. With amounts of nickel of 0.01 mg. or less and an acidity greater than 0.01 molar, the color developed is tinged with yellowish brown instead of clear pink, making comparison with the standards difficult. With a quantity as minute as this, however, it is still possible to make a color comparison with the standards by adjusting the latter to the same degree of acidity as the unknown solutions.

#### DETERMINATION OF NICKEL AS THE CHLORIDE IN CONCENTRATED HYDROCHLORIC ACID

Pure nickel chloride dissolved in concentrated hydrochloric acid gives a yellow solution, the intensity of which is proportional to the amount of nickel present. The maximum color is obtained in about

30 per cent hydrochloric acid or stronger.<sup>8</sup> Hence, all solutions and dilutions must be made with hydrochloric acid of at least 30 per cent strength.

#### Reagents.

1. Hydrochloric acid, sp. gr. 1.19. Must be free of copper, cobalt, iron, and nickel.

2. Concentrated nitric acid, sp. gr. 1.42.

3. Standard nickel solution. Dissolve 4.9554 grams of Cu-, Co-, and Fe-free nickel nitrate,  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , in concentrated hydrochloric acid and evaporate almost to dryness on the water-bath. Add more concentrated hydrochloric acid and again evaporate almost to dryness. Again add more concentrated hydrochloric acid and evaporate. Be certain that all nitric acid has been expelled. Finally, dissolve the residue and make up to 500 cc. with concentrated hydrochloric acid. Thoroughly mix. This solution contains 2 mg. of nickel per cubic centimeter.

**Procedure.**—Dissolve about 0.2 gram of the nickel alloy in concentrated nitric acid, evaporate almost to dryness three times on the water-bath with concentrated hydrochloric acid, dissolve in about 3 drops of hydrochloric acid (1 : 1), transfer to a colorimeter tube, and dilute to 50 or 100 cc. with concentrated hydrochloric acid, depending upon the depth of color. If cobalt is absent, the comparison may be made by the balancing method. A green tinge indicates the presence of cobalt. In this case, use the method of dilution, the solution of sample being diluted with concentrated hydrochloric acid. The standard is given the same green tinge as that of the sample by adding standard cobalt solution. The amount of standard cobalt solution required is a measure of the cobalt content of the sample.

#### Notes.

1. A series of standards is not advisable since in the determination there are usually two variables: (1) the amount of nickel solution used in the pure acid and (2) the amount of cobalt solution added to the nickel solution.

2. The nickel must always be in concentrated hydrochloric acid or the yellow color will turn to green. Also, the cobalt must always be in the concentrated acid or the blue color will turn to pink.

<sup>8</sup> C. Hüttner, *Z. anorg. Chem.*, **86**, 341 (1914).

3. The 0.2 gram sample taken in the above procedure is for alloys and ores containing from 0.1 to 10 per cent of nickel. If the nickel content is less than 0.1 per cent, a larger sample should be used.

4. Copper and iron interfere. The copper is precipitated with hydrogen sulfide, the excess hydrogen sulfide boiled off, the iron oxidized and then precipitated as the basic acetate. Sodium chloride does not interfere. Nitric acid and free chlorine must not be present.

5. Since nitric acid must be removed by repeated evaporations with concentrated hydrochloric acid, the sample should be dissolved in the latter, if possible. Unfortunately, this is usually not possible and nitric acid must be used.

6. The nickel and cobalt may be separated together electrolytically, dissolved and treated as given in the procedure above.



## CHAPTER XXVI

### NITROGEN

#### (Ammonia, Nitrite, and Nitrate)

##### DETERMINATION OF AMMONIA BY NESSLER'S REAGENT

NESSLER'S<sup>1</sup> reagent is an alkaline solution of potassium mercuric iodide,  $K_2HgI_4$ . This reagent reacts with ammonia or ammonium salts to form an orange-colored complex compound of the composition  $HgO \cdot Hg(NH_2)I$ . The reaction is an extremely delicate test for ammonia and ammonium salts. If the solution contains more than a few milligrams of ammonia per 100 cc., a distinct orange precipitate is formed; smaller concentrations produce a yellow to brown colored solution.

##### Reagents.

1. Nessler's reagent.<sup>2</sup> Dissolve 2.5 grams of potassium iodide in 3 cc. of water, add 3.5 grams of mercuric iodide and stir until solution is complete. Then add 100 grams of a 15 per cent solution of potassium hydroxide, mix, allow to settle, and decant the clear supernatant liquid. If the reagent is needed at once, add a little talc and filter through a column of clean, finely divided sand. Keep the solution in the dark.

2. Rochelle salt. Dissolve 50 grams of Rochelle salt,  $KNaC_4H_4O_6$ , in 100 cc. of water and add 5 cc. of the Nessler reagent as a preservative.

3. Standard ammonium chloride solution. Dissolve 0.3141 gram of pure ammonium chloride in water, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 0.1 mg. of ammonia.

**Procedure.**—Add 3 cc. of the Rochelle salt solution and 3 cc. of Nessler's reagent to 100 cc. of the sample. A yellow to brown color

<sup>1</sup> J. Nessler, *Chem. Gaz.*, 1856, 446.

<sup>2</sup> G. Frerichs and E. Mannheim, *Apoth. Ztg.*, 29, 972 (1914).

appears at once. Match the color against a series of standards, by the dilution or balancing method, or by the method of duplication.

A standard for the methods of balancing and duplication may be prepared by adding 20 cc. of the Rochelle salt solution and 20 cc. of Nessler's reagent to 100 cc. of the standard ammonium chloride solution and diluting to a liter. One cubic centimeter of this standard contains 0.01 mg. of ammonia. In case this standard is too concentrated, dilute it to one-fifth or to one-tenth of its strength.

#### Notes.

1. Calcium and magnesium are precipitated by Nessler's reagent and, hence, would interfere with the analysis. They may be removed by precipitating in the usual way and filtering, but it is more convenient to add Rochelle salt which holds them in solution.

2. The yellow to brown color produced by the reaction of ammonia with Nessler's reagent is fairly stable.

3. J. H. Robertson and A. Hissey<sup>3</sup> have recently made an extensive experimental study on the nature of the Nessler color and conclude that it is due to colloiddally dispersed particles in suspension and not in true solution.

#### DETERMINATION OF AMMONIA BY PHENOL AND SODIUM HYPOCHLORITE

When phenol and sodium hypochlorite are added to a solution containing an ammonium salt an intense blue coloration is produced. The reaction is said to be as sensitive as the Nessler test but does not permit as great an accuracy.<sup>4</sup>

#### Reagents.

1. Sodium hypochlorite. Use a dilute solution of NaOCl.

2. Phenol, 4 per cent.

3. Standard ammonium chloride solution. Dissolve 0.3141 gram of purified ammonium chloride in water, dilute to a liter and thoroughly mix. Dilute 10 cc. of this solution to 100 cc. and mix. The diluted solution contains 0.01 mg. of ammonia per cubic centimeter.

**Procedure.**—Place 5 cc. of the sample in a tube, add 1 cc. of dilute sodium hypochlorite solution, 1 cc. of the phenol solution, dilute to

<sup>3</sup> Private communication from Dr. J. H. Robertson.

<sup>4</sup> P. Thomas, *Bull. soc. chim.*, **11**, 796; G. E. Foxwell, *Gas World*, **64**, No. 1654 (Cooking Section), 10 (1916).

10 cc., mix, and heat for 2 minutes by placing the tube in boiling water. Heating brings out the maximum intensity of color. Match the color against a series of standards, or by the methods of balancing and dilution. To prepare a standard suitable for the two latter methods, add 5 cc. of the sodium hypochlorite solution and 5 cc. of the phenol solution to 10 cc. of the ammonium chloride (0.3141 gram  $\text{NH}_4\text{Cl}$  per liter) solution, dilute to a liter, and thoroughly mix. One cubic centimeter of this standard contains 0.001 mg. of ammonia.

#### Notes.

1. Matching the color by the method of duplication is not very satisfactory.<sup>5</sup>
2. Calcium does not interfere with the analysis. A large amount of free acid must be avoided.
3. The above procedure will detect as little as 0.0001 mg. of ammonia in the 5 cc. sample taken.

#### DETERMINATION OF NITRITE BY SULFANILIC ACID AND $\alpha$ -NAPHTHYLAMINE

When a mixture of acetic acid solutions of sulfanilic acid and  $\alpha$ -naphthylamine is added to a solution containing nitrite, a red coloration appears which gradually darkens on standing. The maximum intensity requires several hours and sometimes several days, but a satisfactory color comparison may be made after about 5 minutes, provided the sample and standard are treated at the same time.

#### Reagents.

1. Sulfanilic acid. Add 1 gram of sulfanilic acid to 14.7 grams of glacial acetic acid and 15 cc. of water. Warm until solution is complete and dilute by adding 270 cc. of water. Stir while diluting.
2.  $\alpha$ -Naphthylamine. Add 0.2 gram of  $\alpha$ -naphthylamine to 14.7 grams of glacial acetic acid and 25 cc. of water. Warm until solution is complete and dilute with 300 cc. of water. Stir while diluting.
3. Standard nitrite solution. Dissolve 0.0493 gram of purified sodium nitrite in water and dilute to 100 cc. Thoroughly mix. Add 10 cc. of this solution to 90 cc. of concentrated sulfuric acid and mix. One cubic centimeter of the nitrosyl sulfuric acid contains 0.01 mg. of nitrogen.

<sup>5</sup> F. D. Snell, *Colorimetric Analysis*, p. 120. D. Van Nostrand Co., New York, 1921.

**Procedure.**—Place 20 cc. of the sample in a Nessler tube and 18 cc. of distilled water in a second Nessler tube. Add to the water an amount of standard nitrite solution estimated to be the amount present in the sample. Then to each Nessler tube add 3 cc. of a freshly prepared mixture of the sulfanilic acid and  $\alpha$ -naphthylamine reagents, mix the contents of the tubes, allow to stand 5 minutes and compare their colors. If they are not identical, repeat the analysis, using a standard containing the amount of nitrite estimated to be in the sample. After a little experience, the operator can estimate fairly closely the amount of nitrite to take for the second test.

**Note.**—The nitrous acid converts the sulfanilic acid into the corresponding diazo compound and the latter reacts with the  $\alpha$ -naphthylamine to form a red azo dye.<sup>6</sup> As mentioned above, the formation of the red color requires several hours or even days to reach its maximum intensity but a satisfactory depth of color is obtained in 5 minutes. Hence, by treating the sample and standard at the same time and keeping the conditions (temperature, volume of reagents, etc.) identical, a comparison may be made in 5 minutes after adding the reagents.

#### DETERMINATION OF NITRITE BY $\alpha$ -NAPHTHYLAMINE HYDROCHLORIDE

This method is based upon the color reaction between nitrous acid and a solution of  $\alpha$ -naphthylamine hydrochloride and tartaric acid. It is necessary that the test solution contain not more than 0.15 mg. of nitrous acid per liter, otherwise the reagent will produce a precipitate.<sup>7</sup>

##### Reagents.

1.  $\alpha$ -Naphthylamine hydrochloride reagent. Dissolve 5 grams of  $\alpha$ -naphthylamine hydrochloride in 50 grams of concentrated sulfuric acid containing 445 grams of tartaric acid. This solution is perfectly stable.

2. Standard nitrite solution. Dissolve 0.4926 gram of purified sodium nitrite in water, dilute to a liter, and mix thoroughly. Ten cubic centimeters of this solution are then diluted to a liter and mixed. This solution contains 0.001 mg. of nitrogen per cubic centimeter (corresponding to 0.00328 gram of  $\text{NO}_2$  or 0.00443 gram of  $\text{NO}_3$ ).

<sup>6</sup> Fowler, *Sewage Works Analyses*, p. 64. John Wiley & Sons, New York.

<sup>7</sup> G. Romijn, *Chem. Weekblad*, 11, 115 (1914).

**Procedure.**—Place 50 cc. of the sample in a comparison cylinder, add 1 cc. of the  $\alpha$ -naphthylamine reagent, mix and match the color by the method of duplication, balancing, or dilution. In case the sample contains more than 0.15 mg. of nitrous acid per liter, it must be diluted and an aliquot part taken for analysis. With very dilute samples, 100 cc. may be used for the analysis.

To prepare a standard for the balancing or dilution method, dilute 1 cc. of the standard (containing 0.4926 gram of sodium nitrite per liter) with 800 to 900 cc. of water, add 1 cc. of the reagent, dilute to a liter, and mix thoroughly. One cubic centimeter of this standard contains 0.0001 mg. of nitrogen.

**Note.**—A series of standards is not recommended on account of the delicacy of the test.

#### DETERMINATION OF NITRITE BY METAPHENYLENEDIAMINE

The method is based upon the formation of triaminoazobenzene (Bismarck brown) by the action of nitrous acid on metaphenylenediamine.<sup>8</sup> The color varies from yellow to yellowish brown, depending upon the amount of nitrous acid present.

##### Reagents.

1. Sulfuric acid, 6 N.
2. Metaphenylenediamine. Dissolve 5 grams of metaphenylenediamine in water, make the solution distinctly acid with sulfuric acid and dilute to a liter. In case the solution is not colorless, decolorize with animal charcoal.
3. Standard nitrite solution. Dissolve 0.0493 gram of purified sodium nitrite in water, dilute to 100 cc. and thoroughly mix. Dilute 10 cc. of this solution with 90 cc. of sulfuric acid, sp. gr. 1.84. One cubic centimeter of this nitrosyl sulfuric acid contains 0.01 mg. of nitrogen.

**Procedure.**—Place 100 cc. of the sample in a Nessler cylinder and add a mixture of 2 cc. of 6 N sulfuric acid and 1 cc. of the metaphenylenediamine reagent. Mix and compare against a series of standards prepared with measured quantities of the nitrosyl sulfuric acid diluted to 100 cc. before adding the reagent.

<sup>8</sup> P. Griess, *Ber.*, **11**, 624 (1878).

If it is desired to make the color comparison by the method of balancing or that of dilution, a convenient standard may be prepared by treating 10 cc. of the standard nitrosyl sulfuric acid solution with 2 cc. of the reagent, diluting to 100 cc. and mixing. This gives a standard solution containing 0.001 mg. of nitrogen per cubic centimeter.

Since Bismarck brown is formed very quickly, the method of duplication may also be used.

#### DETERMINATION OF NITRITE BY DIMETHYLANILINE <sup>9</sup>

When a solution of dimethylaniline in dilute hydrochloric acid is added to a solution containing nitrite, a yellow color develops in from 15 to 30 minutes. The color is due to the formation of *p*-nitrosodimethylaniline and its intensity is proportional to the nitrite content of the original solution. Nitrates do not interfere and the reaction will detect 1 part of nitrous acid in 1,000,000 parts of solution.

##### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Dimethylaniline. Dissolve 8 grams of dimethylaniline in 100 cc. of 4 per cent hydrochloric acid solution.
3. Standard nitrite solution. Dissolve 0.0493 gram of pure sodium nitrite in water, dilute to a liter, and mix thoroughly. The solution contains 0.01 mg. of nitrogen per cubic centimeter.

**Procedure.**—Place 50 cc. of the sample in a colorimeter tube, add 1 drop of concentrated hydrochloric acid, 3 drops of dimethylaniline reagent, mix, and in 15 to 30 minutes compare the color against a standard nitrite solution prepared similarly.

**Note.**—The sample and standard should be treated simultaneously to avoid any difference in depth of color due to a difference in the time of standing before comparing.

#### DETERMINATION OF NITRITE BY ANTIPYRIN <sup>10</sup>

This method is based upon the green coloration produced when an acetic acid solution of antipyrin is added to a solution containing nitrite. One part of nitrous acid in 20,000 parts of solution will give a green coloration.

<sup>9</sup> E. H. Miller, *Analyst*, **37**, 345 (1912).

<sup>10</sup> M. C. Schuyten, *Chem. Ztg.*, **20**, 722 (1896).

**Reagents.**

1. Antipyrin. Use a 10 per cent solution in acetic acid. Dilute with water to a 1 per cent solution as needed.

2. Standard nitrite solution. Dissolve 0.0493 gram of pure sodium nitrite in water, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 0.01 mg. of nitrite nitrogen.

**Procedure.**—Add 5 cc. of 1 per cent antipyrin reagent to 5 cc. of the sample and match the green color thus produced against a series of standard nitrite solutions similarly treated.

**Note.**—The presence of heavy metals or of organic matter does not interfere with the reaction, but the green color changes at once to yellow if ferric salts, or free hydrochloric or sulfuric acids are present.

**DETERMINATION OF NITRITE BY ZINC IODIDE STARCH SOLUTION**

When zinc iodide starch solution is added to a solution containing nitrous acid, a blue color develops whose intensity is proportional to the amount of nitrous acid present. The nitrous acid liberates iodine which in turn forms the characteristic starch-iodide blue.

**Reagents.**

1. Sulfuric acid, 6 N.

2. Zinc iodide starch solution. Twenty grams of stannous chloride and 5 grams of starch are added to 100 cc. of water and the mixture boiled for several hours until the starch has been thoroughly disintegrated. A little water is added from time to time to replace that lost by evaporation. Add 2 grams of zinc iodide, dilute to a liter and allow the solution to stand a week or two and decant the clear supernatant liquid as needed.

3. Standard nitrite solution. Dissolve 0.4926 gram of purified sodium nitrite in water, dilute to a liter, and thoroughly mix. Ten cubic centimeters of this solution are diluted to a liter and thoroughly mixed. One cubic centimeter of the dilute solution contains 0.001 mg. of nitrogen (corresponding to 0.00328 gram of  $\text{NO}_2$  or 0.00443 gram of  $\text{NO}_3$ ).

**Procedure.**—Place 50 cc. of the sample in a comparison tube, acidify with 2 cc. of 6 N sulfuric acid, add 4 cc. of the zinc iodide starch solution, and mix. The color of the solution may be matched against a series of standards similarly prepared or by the method of dilution

or balancing. The standard for the latter two methods is prepared by adding 800 to 900 cc. of water to 10 cc. of nitrite solution containing 0.4926 gram of sodium nitrite per liter, then adding 15 cc. of the zinc starch iodide solution, and finally diluting to a liter and mixing.

#### Notes.

1. Matching the colors by the method of duplication is not advisable on account of the time required for the color to develop. If the solutions are placed in the sunlight the color develops more rapidly.

2. The zinc iodide starch method has been estimated to be twenty times more sensitive than the metaphenylenediamine reaction.<sup>11</sup> (See page 310.)

A blue color will appear within 7 minutes if 0.00025 mg. of nitrous acid is present in the 50 cc. sample taken.

#### DETERMINATION OF NITRATE BY PHENOLDISULFONIC ACID <sup>12</sup>

When phenoldisulfonic acid is added to a solution containing a minute quantity of nitrate a yellow coloration is produced, the intensity of which is proportional to the nitrate content. The method is especially adapted to water analysis and has been adopted by the American Public Health Association. The following paragraphs on "Reagents" and "Procedure" have been taken from the A. P. H. A. "Standard Methods."<sup>13</sup>

#### Reagents.

1. Phenoldisulfonic acid. "Dissolve 25 grams of pure white phenol in 150 cc. of pure concentrated sulfuric acid. Add 75 cc. of fuming sulfuric acid (15 per cent  $\text{SO}_3$ ), stir well, and heat for 2 hours at about 100° C."

2. Potassium hydroxide solution. "Prepare an approximately 0.12 N solution, 10 cc. of which will neutralize about 4 cc. of the phenoldisulfonic acid."

3. Standard nitrate solution. "Dissolve 0.7215 gram of pure

<sup>11</sup> Analyst, **39**, 350 (1914).

<sup>12</sup> E. M. Chamot and D. S. Pratt, J. Am. Chem. Soc., **31**, 922 (1909); *ibid.*, **32**, 630 (1910); and H. W. Redfield, *ibid.*, **33**, 366; 381 (1911).

<sup>13</sup> Standard Methods for the Examination of Water and Sewage, 6th ed., p. 20. American Public Health Association, New York, 1925.



recrystallized potassium nitrate in 1 liter of distilled water. Evaporate 50 cc. of this solution to dryness on the water-bath. Moisten the residue quickly and thoroughly with 2 cc. of phenoldisulfonic acid, and rub with a glass rod to insure intimate contact. Dilute to 500 cc. This is the standard solution, 1 cc. of which contains 0.01 mg. of nitrate nitrogen, or 0.04427 mg. of  $\text{NO}_3$ ."

4. Standard silver sulfate solution. "Dissolve 4.397 grams of silver sulfate free from nitrate in 1 liter of water: 1 cc. of this solution is equivalent to 1 mg. of chloride radical."

**Procedure.**—"The alkalinity, chloride, nitrite content, and color of the sample must be first determined. If the sample is highly colored, decolorize it with freshly precipitated aluminum hydroxide. Measure into an evaporating dish 100 cc. of the sample: a smaller volume may be used unless the amount of nitrate is low, and in any case the volume of sample should be such that the nitrate nitrogen does not exceed 1 mg. Add sufficient 0.02 N sulfuric acid nearly to neutralize the alkalinity. Then add to the cold solution sufficient standard silver sulfate solution to precipitate all but about 0.1 mg. of chloride. The removal of chloride may be omitted if the sample contains less than 30 parts per million of chloride. To the mixture add a little aluminum hydroxide, stir very thoroughly, allow to stand for a few minutes, filter and wash with distilled water. Most waters, if heated with silver sulfate solution, suffer an appreciable loss of nitrate nitrogen. Evaporate the filtrate to dryness, add 2 cc. of disulfonic acid solution, rubbing with a glass rod to insure intimate contact. If the residue becomes packed or appears vitreous because of the presence of much iron, heat the dish on the water-bath for a few minutes. Dilute the mixture with distilled water and add slowly a strong solution of potassium hydroxide until the maximum color is developed. Transfer the solution to a Nessler tube, filtering if necessary. If nitrate is present a yellow color will appear. Compare the color with that of standards<sup>14</sup> made by adding 2 cc. of strong potassium hydroxide to various volumes of standard nitrate solution and diluting them to 50 cc. in Nessler tubes; the following volumes of standard nitrate solution are suggested: 0.1, 0.3, 0.5, 0.7, 1.0, 3, 5, 10, 20, 30, 40, 50 cc., yielding standards containing 0.001 to 0.5 mg. nitrogen. These standards may be kept several weeks without deterioration.

<sup>14</sup> D. D. Jackson, *Tech. Quart.*, **13**, 314 (1900); L. M. Kendall and E. H. Richards, *ibid.*, **17**, 277 (1904).

"Standards prepared from tripotassium nitrophenol disulfonate<sup>15</sup> will remain permanent for several years if stored in the dark.

"If nitrite nitrogen is present in excess of 1 part per million, it should be removed by heating the samples a few minutes with a few drops of hydrogen peroxide, free from nitrate, repeatedly added,<sup>16</sup> or dilute potassium permanganate may be added until a faint pink coloration persists; the nitrogen equivalent of the nitrite thus oxidized to nitrate is then subtracted from the final nitrogen reading."

#### Notes.

1. When chlorides are present in the water, Gericke<sup>17</sup> recommends the following modified procedure in order to avoid loss of nitrates, which in the above method is due to the action of acid added to the dry salt after evaporation to dryness: "To the sample, placed in a casserole or beaker, 1.5 cc. of concentrated sulfuric acid are added with constant stirring, then 2 cc. of phenoldisulfonic acid reagent. The casserole is then placed on a water-bath, and most of the solution evaporated at the ordinary temperature of the water-bath. The last part of the evaporation, however, should be performed at a temperature preferably not over 70° C.

"The evaporation should proceed until the original solution is concentrated to a quantity varying from 6 or 7 to 12 or 14 cc. The point to which evaporation must be continued is determined by the amount of nitrates in the original solution; for a low nitrate content a greater concentration of the original solution will be necessary. The proper concentration is determined by the color of the solution, which resembles that of phenoldisulfonic acid, slightly tinged with yellow. This condition will come, and final evaporation be attained at about the time that acid, due to the presence of chlorine, can be detected in the evaporating vapors. The important thing to observe in this modification is to bring about the final evaporation at a relatively low temperature. In no case should the solution be materially colored and turbid, although it may be somewhat darkened. A colored solution will result in an off tint when the alkali is added and will necessarily interfere with the accuracy of the determination. When the evaporation of the solution to its proper concentration has been

<sup>15</sup> Chamot and Pratt, *loc. cit.*

<sup>16</sup> R. R. Tatlock and R. T. Thomson, *J. Soc. Chem. Ind.*, **23**, 428 (1904).

<sup>17</sup> *J. Ind. Eng. Chem.*, **9**, 585 (1917).

accomplished, about 50 cc. or more of water are added; the solution is then neutralized with an alkali, care being taken to avoid the formation of excessive temperature when the acid is neutralized. The solution is then placed in the colorimeter and compared with a standard previously prepared. . . . In cases of very low nitrate and high salt content, evaporation of the solution to its proper concentration should be performed at a much reduced temperature and preferably under partial vacuum, in order to reduce the action of acids on the salts."

2. Nichols<sup>18</sup> recommends adding a saturated solution of ammonium chloride after neutralization of the excess acid and before the final dilution. The ammonium salt holds the magnesium in solution and, hence, makes it unnecessary to filter in the absence of ferric hydroxide.

#### DETERMINATION OF NITRATE BY DIPHENYLBENZIDINE

It is well known that a solution of diphenylamine in sulfuric acid is a delicate reagent for detecting small amounts of nitrates,<sup>19</sup> a deep blue solution being formed. As a quantitative reagent, however, it is not satisfactory on account of the difficulty in controlling the reaction so as to give uniform results. Diphenylbenzidine, on the contrary, may be similarly employed as a quantitative reagent.

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84. Be certain that the acid is free of nitrous and nitric acids.

2. Diphenylbenzidine. Prepare according to Wieland<sup>20</sup> and purify by recrystallization until the correct melting point (242°) is obtained.

Dissolve 0.5 gram of the purified diphenylbenzidine in 50 cc. of pure sulfuric acid. A fresh solution should be made up about once a week, since such solutions gradually turn blue.

3. Standard nitrate solution. Dissolve 0.3608 gram of pure potassium nitrate in water, dilute to a liter, and thoroughly mix. Dilute 10 cc. of this solution to 100 cc. and mix. One cubic centimeter of the diluted solution contains 0.005 mg. of nitrogen.

<sup>18</sup> J. Ind. Eng. Chem., **9**, 586 (1917).

<sup>19</sup> E. Kopp, Ber., **5**, 284 (1872).

<sup>20</sup> Ber., **46**, 3300 (1913).

**Procedure.**—The sample should contain between 0.0005 and 0.005 mg. of nitrogen. The volume of the sample is adjusted to 5 cc., if necessary, and to it are added 12 cc. of sulfuric acid, sp. gr. 1.84, and the solution placed in a cold water-bath to cool. When cool, add 3 cc. of the diphenylbenzidine reagent, mix gently with a glass rod, let stand 10 minutes, and match the color against a series of standards similarly prepared along with the sample, using measured amounts of the standard nitrate solution. Let the solutions stand an hour at a uniform temperature and again match the color of the sample.

#### Notes.

1. The temperature, proportion of the nitrate solution and reagents, and the time allowed to elapse after mixing the reagents with the solution should be kept as uniform as possible and the same for both sample and standard.
2. When both nitrite and nitrate are to be determined, oxidize with potassium permanganate and estimate the total nitrogen as nitrate. By subtracting the nitrate nitrogen from the total nitrogen, the nitrite nitrogen is obtained.

#### DETERMINATION OF NITRATE BY REDUCTION AND NESSLERIZATION

This is one of the methods for nitrate adopted by the American Public Health Association. The method of preparing the reagents and the procedure are taken from "Standard Methods for the Examination of Water and Sewage," 6th ed., p. 21, New York, 1925.

#### Reagents.

1. Sodium or potassium hydroxide. Dissolve 250 grams of the hydroxide in 1.25 liter of distilled water. Add several strips of aluminum foil and allow the evolution of hydrogen to continue overnight. Concentrate the solution to 1 liter by boiling.

2. Aluminum foil. Use strips of pure aluminum about 10 cm. long, 6 mm. wide, and 0.33 mm. thick, weighing about 0.5 g.

**Procedure.**—"To 100 cc. or less of the sample in a 300 cc. casserole add 2 cc. of the hydroxide solution and concentrate by boiling to about 20 cc. Pour the contents of the casserole into a test tube about 16 cm. long and 3 cm. in diameter, of approximately 100 cc.

capacity. Rinse the casserole several times with nitrogen-free water and add the rinse water to the liquid already in the tube, thus making the contents of the tube approximately 75 cc. Add a strip of aluminum foil; close the tube by means of a rubber stopper through which passes a bent glass tube about 5 mm. in diameter. Put the shorter arm of the tube through flush with the lower side of the rubber stopper and let the longer arm extend below the surface of distilled water in another test tube. This apparatus serves as a trap through which evolved hydrogen escapes freely. The small amount of ammonia escaping into the trap may be neglected. Allow the action to proceed for a minimum period of 4 hours, or overnight. Pour the contents of the tube into a distilling flask, dilute with 250 cc. of ammonia-free water, distill and collect the distillate in a 200 cc. flask, and Nesslerize an aliquot part. If the supernatant liquid in the reduction tube is clear and colorless, the solution may be diluted to a definite volume and an aliquot part Nesslerized without distillation."

## REFERENCES

1. A. Hazen, *Chem. News*, **64**, 162 (1891).
2. E. Bartow and J. S. Rogers, *Am. J. Pub. Hygiene*, new ser., **5**, 536 (1909); also *Univ. Ill. Bull.*, **7**, No. 2 (Water Survey Series, 7), 14 (1909).

DETERMINATION OF NITRATE BY BRUCINE<sup>21</sup>

When brucine is added to a solution of a nitrate in the presence of sulfuric acid the solution turns an orange-red color but changes to yellow. The intensity of the yellow color is proportional to the nitrate content of the solution. If a nitrite is present, the solution must be made sufficiently acid, otherwise the nitrite will produce a yellow color.

The method is used in the analysis of water, sewage, acids, salts, etc.

## Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Brucine. Use a concentrated solution.
3. Standard nitrate solution. Dissolve 0.1872 gram of pure potassium nitrate in water, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 0.1 mg. of  $N_2O_5$ , 0.115 mg. of  $NO_3$ , or 0.026 mg. of nitrogen.

<sup>21</sup> L. N. Winkler, *Chem. Ztg.*, **23**, 454 (1899); **25**, 586 (1901).

**Procedure.**—The sample taken for analysis should contain between 0.01 and 0.2 mg. of nitric acid. If a solid, it is dissolved in water and diluted to about 20 cc., if a liquid, a measured volume of the substance is adjusted to about 20 cc. To the sample solution in a Nessler cylinder add 1 cc. of a concentrated brucine solution and 30 cc. of sulfuric acid, sp. gr. 1.84, taking care to add the acid slowly down the side of the tube so as not to bring the solution to a boil, thereby causing a loss of nitric acid. The sulfuric acid brings out the color, at first an orange yellow, then a sulfur yellow. The color comparison is most conveniently made by the method of duplication. For this, use 15 cc. of water and 5 cc. of concentrated sulfuric acid as the blank. This should be colorless; if not, reject it, and make up a blank with purified sulfuric acid.

#### Notes.

1. If the solutions are allowed to cool, the color change will be slow. In this case, gently warm the solutions over a flame.

2. The test solution must contain at least 2 parts of sulfuric acid to 1 of water, otherwise nitrous acid will produce a color. If it is desired to determine both nitrous and nitric acid, adjust the solution so that it contains one part of sulfuric acid to two parts of water.

3. If the sample contains ferrous iron or organic matter, these must be oxidized with permanganate solution. The permanganate solution is added, drop by drop, until the palest pink color remains in the sample solution. It must be remembered that this treatment will also oxidize any nitrous acid present. Hence, if it is desired to obtain both acids, the nitrous acid must be obtained in a separate sample. Calculate the nitrous acid to nitric acid and subtract the result from the total nitric acid found in the oxidized sample. This gives the amount of nitric acid present in the original sample.

4. If the sample under examination is sulfuric acid, then 10 cc. of water and 10 cc. of acid are used instead of the 30 cc. of concentrated sulfuric acid used for other substances.

#### DETERMINATION OF NITRATE BY PYROGALLOL<sup>22</sup>

This method is suitable for a quick and approximate estimate of the amount of nitrate in sewage effluent and in water. With practice, the operator can estimate fairly accurately the amount of nitrate present.

<sup>22</sup> Report of the Royal Commission on Sewage Disposal, Vol. IV, Part V., p. 23.

**Reagents.**

1. Sulfuric acid, sp. gr. 1.84.
2. Pyrogallol.
3. Sodium chloride, powdered.
4. Standard nitrate solution. Dissolve 0.1872 gram of potassium nitrate in water, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 0.1 mg. of  $\text{N}_2\text{O}_5$  or 0.115 mg. of  $\text{NO}_3$ .

**Procedure.**—Place 10 cc. of the sample in a test tube, add about 0.2 gram of pyrogallol and thoroughly mix. Then introduce 2 cc. of sulfuric acid, sp. gr. 1.84, so that it runs down the side of the tube and forms a layer at the bottom. Before allowing the acid to flow, have the tip of the pipette inserted just below the surface of the liquid. Next add about 0.1 gram of powdered sodium chloride. This causes an effervescence at the juncture of the two liquid layers and the formation of a purple ring. The amount of nitrate is proportional to the intensity and size of the purple ring. After experience, the operator can guess fairly well the amount of nitrate present. This is then checked against a standard containing the amount of nitrate estimated to be in the sample.

**DETERMINATION OF NITRATE BY STRYCHNINE SULFATE****METHOD OF SCALES AND HARRISON<sup>23</sup>**

A specially prepared strychnine sulfate reagent is mixed with the test solution and concentrated sulfuric acid then added. Upon mixing, a rose color develops if nitrate is present. With very weak solutions of nitrates it may require about 30 minutes for the color to develop, but stronger ones give a color in a few minutes.

**Reagents.**

1. Sulfuric acid, sp. gr. 1.84.
2. Reduced strychnine. It is better to purchase the strychnine sulfate for this reagent in 5 gram bottles and to keep it well protected from contact with the air. Equal volumes of a 0.5 per cent solution of strychnine sulfate in concentrated hydrochloric acid and a 0.1 per cent solution of mercuric chloride in distilled water are mixed. Twenty-

<sup>23</sup> Ind. Eng. Chem., **16**, 571 (1924); cf. Denigès, Bull. soc. chim. [4], **9**, 544 (1911).

five cubic centimeters of this mixture are poured cautiously over 1 gram of magnesium powder in a 300 cc. Erlenmeyer flask. The reaction is almost violent. Three or more flasks are prepared in this way, then combined, and when cool the liquid is filtered or decanted and is ready for use. It is better to make several reductions in this way and combine them than to make up the reagent in larger quantity, as it is safer and at the same time neutralizes any variations in the reduction. The reduced strychnine should be used within a few hours.

3. Standard nitrate solution. Dissolve 6.07 grams of pure sodium nitrate in water, dilute to a liter, and thoroughly mix. One cubic centimeter of this solution contains 1 mg. of nitrate nitrogen. Dilute this stock solution to any desired concentration.

**Apparatus.** A dark box is employed, made in the form of a solid block of wood 38 cm. square, 15 cm. thick, with 100 holes 2.5 cm. in diameter and 11.5 cm. deep, to accommodate ten rows of tubes. It has a hinged cover and is finished in black. The tubes should be of uniform size and color. Very satisfactory dimensions are  $13 \times 1.5$  cm. A burette of 100 cc. capacity with a cock and tip of 4 mm. bore is used for the rapid delivery of concentrated sulfuric acid.

**Procedure.**—Extract the substances containing nitrate in any satisfactory way. Dilute the extracts in varying amounts, as 50 times, 100 times, 300 times, etc. Run 1 cc. of the reagent into each tube in the first two rows in the dark box.

Measure out into the first row 5 cc. quantities of known standard nitrate solutions, varying consecutively in concentration, as from 0.01 mg. to 0.10 mg. nitrogen as nitrate per liter. Into the next row measure out 5 cc. quantities of the lowest dilution of the first nine extracts, running 5 cc. of water only into the tenth tube. Now, add as rapidly as safety will permit exactly 5 cc. concentrated sulfuric acid to all twenty tubes, avoiding any evolution of gas, then agitate immediately by pouring each solution carefully into another tube, and quickly replace it in the box. Run the reagent into the next two rows and proceed as above, using this time the next highest dilution of the unknowns of the first set, making the tenth tube one of water only, as before.

Prepare the next two rows similarly, starting with new unknowns or higher dilutions of the previous ones as the case may warrant, and continue, always completing two rows at a time. Standards and unknowns must be run parallel in this way, as it is of the utmost



importance to compare unknowns and standards to which the sulfuric acid has been added at the same time.

The colors may be read as soon as they develop, which should be but a few minutes for the stronger ones, and not more than half an hour for the weaker ones. The tubes darken in color the longer they stand, if not exposed to light, but are still comparable. Light causes the color to fade.

The unknowns are compared with the standards, the color being observed either across or down through the tubes. The strength of nitrate in the tube of standard which checks with the unknown is taken for the reading,  $R$ . This, multiplied by the dilution,  $D$ , of the unknown gives the corresponding reading for an undiluted sample. This reading is in milligrams per liter (parts per million). To convert to percentage, divide by 1,000,000 : 100, i.e., 10,000; thus the formula  $\frac{R \times D}{10,000}$  gives the per cent nitrogen as nitrate in the undiluted extract, where  $R$  is the reading in milligrams per liter, and  $D$  is the dilution.

#### Notes.

1. The addition of a small quantity of lead, zinc, or mercuric chloride increases the sensitiveness of the strychnine reagent very much. The 0.05 per cent of mercuric chloride used above gives a reagent sensitive to 1 part in 100 millions. Without the chloride, the sensitivity is 1 part in 5 millions. Instead of 0.05 per cent of mercuric chloride, 0.5 per cent of zinc chloride or 0.001 per cent of lead chloride may be used. It is possible the sensitizing action of the mercuric and zinc chlorides may be due, in part at least, to a minute quantity of lead chloride present as an impurity.

2. Great care must be taken in reducing the strychnine sulfate in order to obtain the same reagent each time. Zinc is an unsatisfactory reducing agent because it usually contains a small amount of lead as an impurity. The presence of the lead gives a reagent that produces a color with distilled water. Magnesium is used because it can be obtained comparatively pure, is a vigorous reducing agent, and when used as directed gives a reagent that produces no color with distilled water.

## CHAPTER XXVII

### OXYGEN

#### (Free Oxygen, Hydrogen Peroxide, and Water)

#### DETERMINATION OF DISSOLVED OXYGEN IN WATER BY CUPROUS AMMONIUM CHLORIDE

##### METHOD OF RAMSAY AND HOMFRAY

WHEN ammonium hydroxide is added to water containing a little solid cuprous chloride, the latter dissolves, owing to the formation of cuprous ammonium chloride, a soluble and colorless salt. If dissolved oxygen is present, the cuprous salt is at once oxidized to the cupric condition and the cupric ions immediately form the complex cupric ammonia ions,  $[\text{Cu}(\text{NH}_3)_4]^{++}$ , which color the solution blue. Only a very small amount of cuprous chloride is sufficient to remove all the oxygen dissolved in the water and the intensity of the blue ammoniacal solution is an indirect measure of the amount of free oxygen originally present.

The apparatus used is similar to Mill's colorimeter and was devised by Ramsay and Homfray<sup>1</sup> as a simple, rapid, and fairly approximate method of estimating dissolved oxygen in effluents, in rivers, and in drinking waters. Provision is made for transference of the sample water from the collecting bottle to the colorimeter tube without the water coming in contact with air. The usual precautions in collecting the sample must, of course, be taken.

#### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Ammonium hydroxide, sp. gr. 0.90.
3. Cuprous chloride. Warm a solution of cupric chloride with scrap copper and decant the liquid into water. A white precipitate

<sup>1</sup> J. Soc. Chem. Ind., **20**, 1071 (1901); see also Rideal and Stewart, *Analyst*, **26**, 141 (1901); Rideal, *ibid.*, **26**, 196.

of cuprous chloride appears. Filter off the precipitate, wash it first with hot water, then with alcohol, and finally with ether. Dry the salt with the aid of a suction pump and transfer it to a stoppered black test tube for protection against light and moisture. The salt must not be used unless it is pure white, otherwise it may contain some cupric chloride.

**Apparatus.**—*A* and *B* in Fig. 51 are two glass comparison-tubes, each 12 ins. long and 2 ins. in diameter; they are closed with movable caps, *E*; *d* and *d'* are opal glass disks supported by brass wires, which

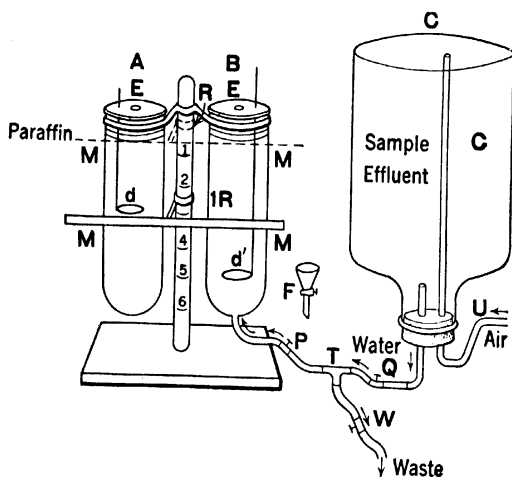


FIG. 51.—Ramsay and Homfray Colorimeter for Oxygen.

slide with friction through holes in the caps, and hold the disks in any required position.

The tubes are supported on a stand with a vertical brass stem, which is graduated in inches and tenths. The levels of the disks and of the liquids in the tubes are read off against this scale with the help of the horizontal leveling rod, *MM*, which slides along the vertical brass stem and can be fixed at any point by the clamp *R*. The bottle *C* contains the sample water or effluent.

Just before the experiment, it is unstoppered and fitted with rubber corks and tubes as shown. Care must be taken that no air bubbles are included; the bottle is then inverted, supported on a tripod stand,

and connected by thick rubber tubing through a T-piece with *B*, as shown.

*To Fill B from C.*—The clamp *R* is set at 0.3 division on the vertical brass stem, reading by the bottom of *R*. Open screw clips *Q*, *W*, and *U*, and run off some water till all air is displaced from *C* to *W*. Close *Q*. Remove cap from *B* and pour in some paraffin. Replace cap and disk. Run off enough paraffin through *W* to displace air from tube *B* to T-piece, leaving a depth of 1 to 2 cm. in *B*. Close *W* and open the other clips. Water is thus allowed to flow quietly into the colorimeter tube *B* until level with the rod *MM*. The paraffin layer rises above the water, acting as a liquid stopper and preventing absorption of atmospheric oxygen for a considerable time.

*To Fill A with Standard for Comparison.*—All corrections are eliminated by using as a standard distilled water saturated with air, both being at the laboratory temperature, which must be noted. The water is shaken in an open flask till air bubbles are seen, and is allowed to stand until they have disappeared. It is poured quietly into a colorimeter tube *A* till its level reaches *MM*. After making sure that no air bubbles are entrapped below the disk, a layer of paraffin is poured on to the water. The ratio of concentrations of oxygen in the waters in *A* and *B* can now be measured. The clamp, *R*, is moved to zero. *MM* is then the final level.

*Addition of Reagents.*—A little of the cuprous chloride powder is poured into a small tap funnel, *F*, and covered with concentrated hydrochloric acid; a dark brown solution results. Some is then run into *A* and *B*, avoiding air bubbles. A white precipitate forms and aqueous ammonia is at once added from a pipette, till the liquid in both tubes reaches *MM*; on stirring gently with the disks, solution takes place at once; the cuprous ammonium double salt is soluble and colorless, but some of it is oxidized to the cupric condition at the expense of all the oxygen dissolved in the water. A very small quantity of this powder suffices to insure the removal of all the dissolved oxygen, but some excess does not interfere if sufficient ammonia is at once added. If any white precipitate remains, it turns yellow, for it is very sensitive to light and interferes with the readings. The solutions (if colored) should be of a pure grayish blue. A greenish color indicates that an insufficient amount of ammonia has been added, unless the sample water itself was colored or opalescent.

The colorimetric estimation consists in determining the ratio of the

concentrations of the colored cupric ammonia complex ions in the two tubes.

*The Determination.*—The observer, looking through a hole in the cap of each tube, adjusts the levels of the opal glass disks,  $d$ ,  $d'$ , so that, when seen from above through the liquids, the intensity of color appears the same in both. The supports of the tubes are hinged to move in a horizontal plane, and their distance apart is regulated by the observer so that he can look down both tubes at once.  $MM$  is made to coincide with the level of each disk in succession, and readings are taken on the vertical brass stem. This gives the depth of  $d$  below the level of the liquid at the zero.

Let the readings be  $p$  and  $q$  in tubes  $A$  and  $B$ , respectively.

Intensity of color, i.e., the total selective absorption of light in traveling through the lengths  $2p$ ,  $2q$ , respectively, of the solutions is the same by adjustment.

But this absorption depends only on the number of molecules of solute encountered, the reflecting disk being white and the solvent colorless.

Hence, in the two solutions,

$$pc = qc',$$

where  $c$   $c'$  are concentrations in  $A$  and  $B$ ,

$$\therefore \frac{c'}{c} = \frac{p}{q}.$$

Thus,  $p/q \times 100$  expresses the percentage oxygen saturation compared to water saturated at known temperatures.

#### DETERMINATION OF DISSOLVED OXYGEN IN WATER BY CUPROUS AMMONIUM CHLORIDE

METHOD OF FRANKFORTER, WALKER, AND WILHOIT<sup>2</sup>

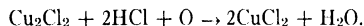
Like the preceding method of Ramsay and Homfray, this determination depends upon the change in color of cuprous ammonium chloride when brought in contact with oxygen. The apparatus devised by Ramsay and Homfray is very simple and rapid, and gives fairly approximate results in estimating dissolved oxygen in water. For accurate determinations a perfectly colorless solution of cuprous ammonium chloride must be used. Such a solution is difficult to pre-

<sup>2</sup> J. Am. Chem. Soc., **31**, 35 (1909).

pare and to keep. Frankforter and his co-workers constructed an apparatus in which a solution of the pure, colorless cuprous double salt can be prepared and kept ready at all times for use. Their apparatus is more elaborate and requires more skill to manipulate than the one of Ramsay and Homfray, but with a little experience accurate determinations of oxygen may be easily and rapidly made.

### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Ammonium hydroxide, sp. gr. 0.90.
3. Oxygen-free water.
4. Copper wire.
5. Standard copper solution. Dissolve 1.1355 grams of pure copper in aqua regia, evaporate off the excess of acid, dissolve the residue in water, make up to one liter, and thoroughly mix. One cubic centimeter of this solution is equivalent to 0.1 cc. of oxygen as indicated by the following equation:



The ratio between the weight of copper and oxygen in this equation is 127.14 : 16. Substituting the weight of 0.1 cc. oxygen under standard conditions we have the equation

$$127.14 : 16 = x : 0.00014290$$

$$x = \frac{127.14 \times 0.00014290}{16} = 0.0011355,$$

the equivalent of copper in 0.1 cc. or 1.1355 grams in a liter.

**Description of Apparatus.**—Frankforter, Walker, and Wilhoit give the following description of their apparatus, Fig. 52: *A* is a burette connected, by means of a stopcock *B*, with *C*, a reservoir for preparing and keeping cuprous chloride. *E*, a second burette for supplying ammonia, is connected with *C* and *F* by means of a three-way cock, *D*. *F* is a mixing bulb for the preparation of pure cuprous ammonium chloride. *G* is a three-way cock connecting *F*, by means of a capillary tube, with the colorimeter *K*, and *H* is a reservoir for holding the sample of water. Reservoir *H* contains a tube, *N*, which extends nearly to the top of the reservoir, the lower end of which is connected with a Kipp hydrogen generator for replacing the water, as it is drawn out, with hydrogen.

*K* is arranged with a stopcock, *I*, and a cork through which passes a large tube *L*, the lower end of which is closed by a disk firmly cemented to the tube. Comparisons with the standard are made by looking down through this tube. *M* is an outlet for air and water in completely filling the colorimeter. *K'* is a similar colorimeter tube for making comparisons. To charge the apparatus, copper wire is first introduced into *C* through *A* and *B*, the opening in the stopcock *B* being large enough to admit pieces of copper wire. *C* is then filled with a saturated solution of cuprous chloride in hydrochloric acid and the whole allowed to stand until any cupric chloride, which may have

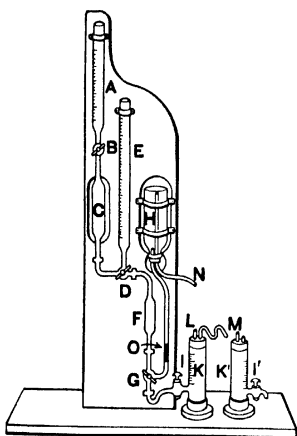


FIG. 52.—Frankforter, Walker, and Willhoit Colorimeter for Oxygen.

been formed in filling the reservoir, has been reduced. *A* is then filled with concentrated hydrochloric acid, to be added as the cuprous chloride is drawn out through *D* into *F*, where it is converted into the double salt. *F* is filled with oxygen-free water from *H* by opening the three-way cocks *G* and *D*. When the water reaches *D*, *G* and *D* are closed, *E* is filled with ammonia, and the apparatus is ready for making the double salt. *C* is now connected with *F*, and *B* is cautiously opened, when cuprous chloride will pass into *F* as soon as *G* and *I* are opened. By noting the height of the acid in *A*, the quantity of saturated cuprous

chloride introduced into *F* may be accurately measured off. In charging the apparatus, 2 cc. of the chloride and 8 cc. of ammonia are run in, the capacity of *F* being about 10 cc. This amount of ammonia is sufficient to convert all the chloride into the double salt. Two cubic centimeters of the reagent are used in each determination. It is made by mixing 0.5 cc. of the cuprous chloride and 1.5 cc. of the strong ammonia.

The manipulation is as follows: *H* is completely filled with the sample of water, corked, placed in position on the stand and connected with the apparatus at *O*. *N* is connected with a hydrogen generator.

By opening *I*, connecting *H* with *K* by means of the three-way cock, *G*, the colorimeter tube *K* is completely filled with the sample. Care must be taken that the small bubbles of air which cling to the cork are removed. The colorimeter tubes *K* and *K'* are so constructed that when the corks are placed in position, they each hold exactly 102 cc. After the colorimeter has been carefully filled with the sample, 2 cc. of the cuprous ammonium chloride from *F* are introduced. This is accomplished by connecting *F* with *K*, opening *B* and cautiously opening *D*. When 0.5 cc. of the reagent has been introduced into *K*, *B* is closed and *D* is turned so as to connect *E*, containing ammonia, with *K*. 1.5 cc. of the reagent are introduced into *K*, making a total of 2 cc. If this is done with sufficient speed, none of the reagent passes out of the tube *M*, and there is left in the colorimeter just 100 cc. of the sample of water and the 2 cc. of reagent. *G* and *I* are now closed and the colorimeter which is connected with the apparatus by a flat ground joint and rubber tube, may be removed for comparison. As soon as the cuprous solution comes in contact with the oxygen in the water, the blue color appears, the intensity of which is measured by the amount of free oxygen present in the water.

The comparison is made by means of *K'*, a second colorimeter tube, and a standard solution of cupric chloride of such strength that 1 cc. of the solution will be equivalent to 1 cc. of oxygen in a liter of water when the quantity of water taken for analysis is 100 cc.

**Procedure.** In the regular analysis, the sample of water is treated as indicated above and the color produced by the cuprous reagent is matched by a known quantity of standard cupric solution. This is accomplished by placing a known amount of the standard solution in the second colorimeter tube with sufficient ammonia to convert the copper into the double salt, and making up to 100 cc. with water. An excess of the standard is taken so that the color is deeper than that of the sample. The standard solution is now drawn off from the stopcock *I'* until the shade of color in the two tubes is the same. From the quantity of standard solution necessary to match the color in *K*, the amount of oxygen may be easily determined. When 100 cc. of water are taken, the number of cubic centimeters of standard will represent directly the number of cubic centimeters of oxygen in a liter of water.

It is necessary, in making more than a single determination, to fill



the space in *H* occupied by the sample drawn off for analysis by some gas which does not contain oxygen. Nitrogen gives good results but it is too difficult to obtain in pure form to be satisfactory. Carbon dioxide does not seem to give satisfactory results. Hydrogen is satisfactory, although the oxygen seems to diminish if the water remains very long in contact with hydrogen.

#### DETERMINATION OF OXYGEN BY "ADUROL"

This method is based upon the coloration produced by oxygen in water containing a small quantity of "adurol," ammonia and ammonium chloride. "Adurol" is a derivative of quinol (*p*-dihydroxybenzene) and is sold as a photographic developer.

The results by the "adurol" method are not as accurate as those obtained by the cuprous chloride methods.

##### Reagents.

1. Ammonium hydroxide, 6 N, containing about 20 per cent ammonium chloride.
2. "Adurol," or a mixture of 1 part of "adurol," 3 parts of Rochelle salt,  $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ , and 6 parts of borax (previously heated for several hours at  $50^\circ \text{C}$ .).

**Procedure.**—Add to a 50 or 100 cc. sample of water a pinch of "adurol" and 0.5 cc. of 6 N ammonium hydroxide containing about 20 per cent ammonium chloride. The sample should be in a flat-sided bottle of clear glass. The "adurol" and ammonia reagent sink to the bottom and the air above the sample is displaced with carbon dioxide. The bottle is then stoppered and shaken. In this way the absorption of oxygen in the air above the sample is prevented. A series of standards is prepared at the same time the sample is treated. The amount of oxygen in the standards is varied by mixing measured volumes of boiled water with water saturated with oxygen. The air above the standard solutions must be displaced with carbon dioxide before shaking them with the reagents.

**Note.**—Comparison by the method of dilution is unsatisfactory.

TABLE XXVI<sup>1</sup>

SOLUBILITY OF OXYGEN IN FRESH WATER AND IN SEA WATER OF STATED DEGREES OF SALINITY AT VARIOUS TEMPERATURES WHEN EXPOSED TO AN ATMOSPHERE CONTAINING 20.9 PER CENT OF OXYGEN UNDER A PRESSURE OF 760 MM.\*

(Calculated by G. C. Whipple and M. C. Whipple from Measurements of C. J. J. Fox)<sup>2</sup>

Temperature, Degrees C.	Chloride in Sea Water (Parts per million)					Difference per 100 Parts per Million Chloride. Parts per million
	0	500	10000	15000	20000	
	Dissolved oxygen in parts per million					
0	14.62	13.79	12.97	12.14	11.32	0.0165
1	14.23	13.41	12.61	11.82	11.03	.0160
2	13.84	13.05	12.28	11.52	10.76	.0154
3	13.48	12.72	11.98	11.24	10.50	.0149
4	13.13	12.41	11.69	10.97	10.25	.0144
5	12.80	12.09	11.39	10.70	10.01	.0140
6	12.48	11.79	11.12	10.45	9.78	.0135
7	12.17	11.51	10.85	10.21	9.57	.0130
8	11.87	11.24	10.61	9.98	9.36	.0125
9	11.59	10.97	10.36	9.76	9.17	.0121
10	11.33	10.73	10.13	9.55	8.98	.0118
11	11.08	10.49	9.92	9.35	8.80	.0114
12	10.83	10.28	9.72	9.17	8.62	.0110
13	10.60	10.05	9.52	8.98	8.46	.0107
14	10.37	9.85	9.32	8.80	8.30	.0104
15	10.15	9.65	9.14	8.63	8.14	.0100
16	9.95	9.46	8.96	8.47	7.99	.0098
17	9.74	9.26	8.78	8.30	7.84	.0095
18	9.54	9.07	8.62	8.15	7.70	.0092
19	9.35	8.89	8.45	8.00	7.56	.0089
20	9.17	8.73	8.30	7.86	7.42	.0088
21	8.99	8.57	8.14	7.71	7.28	.0086
22	8.83	8.42	7.99	7.57	7.14	.0084
23	8.68	8.27	7.85	7.43	7.00	.0083
24	8.53	8.12	7.71	7.30	6.87	.0083
25	8.38	7.96	7.56	7.15	6.74	.0082
26	8.22	7.81	7.42	7.02	6.61	.0080
27	8.07	7.67	7.28	6.88	6.49	.0079
28	7.92	7.53	7.14	6.75	6.37	.0078
29	7.77	7.39	7.00	6.62	6.25	.0076
30	7.63	7.25	6.86	6.49	6.13	.0075

\* Under any other barometric pressure,  $B$ , the solubility can be obtained from the corresponding value in the table by the formula:

$$S' = S \frac{B}{760} = S \frac{B'}{29.92} \text{ in which } S' = \text{Solubility at } B \text{ or } B',$$

$S$  = Solubility at 760 mm. or 29.92 inches,

$B$  = Barometric pressure in mm.,

and  $B'$  = Barometric pressure in inches.

<sup>2</sup> Standard Methods of Water Analysis, 6th ed., p. 62. American Public Health Association, New York, 1925.

<sup>1</sup> C. J. J. Fox, Trans. Faraday Soc., 8, 68-87 (1909); G. C. Whipple and M. C. Whipple, J. Am. Chem. Soc.,

**DETERMINATION OF HYDROGEN PEROXIDE BY ITS OXIDIZING  
ACTION ON FERROUS IRON**

This method is based upon the oxidation of ferrous iron by hydrogen peroxide and subsequent determination of the ferric iron by potassium thiocyanate.

**Reagents.**

1. Sulfuric acid, 6 N.
2. Potassium thiocyanate, 5 per cent.
3. Standard ferrous iron solution. Dissolve 10.534 grams of pure ferrous ammonium sulfate,  $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , in freshly boiled distilled water, add 5 cc. of sulfuric acid, sp. gr. 1.84, and dilute to a liter with boiled distilled water. Mix thoroughly. One cubic centimeter of this solution contains 1.5 mg. of ferrous iron. A little of the solution must be tested for ferric iron by adding a few drops of potassium thiocyanate solution. Should any ferric iron be present, it must be reduced with hydrogen sulfide or by adding a little zinc. The reagent must be slightly acid and free from reducing agents before being used.
4. Standard ferric iron solution. Dissolve 14.164 grams of pure ferric ammonium alum,  $\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ , in water, add 2 cc. of sulfuric acid, sp. gr. 1.84, dilute to a liter with water and thoroughly mix. One cubic centimeter of this solution contains 1.64 mg. of ferric iron, which is equivalent to 0.5 mg. of hydrogen peroxide.

**Procedure.**—A 10 cc. sample is placed in a Nessler tube, 1 cc. (or more) of the standard ferrous iron solution added and then 1 cc. of the potassium thiocyanate solution. Mix gently and compare at once against a standard prepared by adding potassium thiocyanate to the standard ferric iron solution. The thiocyanate must be added to sample and standard at the same time.

An alternative method is to add freshly standardized hydrogen peroxide, drop by drop, to some of the ferrous iron solution (suitably diluted) containing 1 cc. of the potassium thiocyanate solution until the color matches that of the sample treated as directed in the first paragraph. The amount of hydrogen peroxide in the sample is then obtained directly from the burette reading.

**Note.**—Read the notes given under the thiocyanate method for the determination of iron, pages 234 to 236.

## REFERENCES

1. F. D. Snell, *Colorimetric Analysis*, p. 133. D. Van Nostrand Co., New York, 1921.
2. H. N. Stokes and J. R. Cain, *J. Am. Chem. Soc.*, **29**, 411 (1907).
3. A. Rogai, *Staz. sper. agrar. ital.*, **67**, 659 (1914), through *Ann. chim. applicata.*, **2**, 341.

DETERMINATION OF HYDROGEN PEROXIDE BY  
AMMONIUM MOLYBDATE

This method is based upon the yellow color produced by the action of hydrogen peroxide on a molybdate in acid solution.

**Reagents.**

1. Citric acid, 5 per cent.
2. Ammonium molybdate, 10 per cent.
3. Permanent color standard. Dissolve 0.4 gram of potassium chromate and dilute to a liter. See Note 4.

**Procedure.**—Place 30 cc. of water in a 50 cc. volumetric flask, add 10 cc. of 5 per cent citric acid, 1 cc. of the unknown dilute hydrogen peroxide, and mix. Then add, drop by drop, 1 cc. of a 10 per cent ammonium molybdate solution, dilute to the mark with water, and mix thoroughly. Transfer the solution to a colorimeter tube and match against the standard color solution.

**Notes.**

1. It is important that all reagents be added in the order given.
2. The acid and molybdate concentrations may be varied considerably without affecting the color. Likewise, changes in room temperature do not alter the color.
3. Nitric acid could be used instead of citric, but the color is less than half as intense as that developed when citric acid is employed.
4. Isaacs<sup>5</sup> obtained the following results with the above procedure, using a standard prepared with 99.4 per cent pure chromate and applying the formula  $x = \frac{0.05467}{\text{unknown reading}}$ , where  $x$  is equal to the number of grams of peroxide in the 50 cc. of solution:

<sup>5</sup> *J. Am. Chem. Soc.*, **44**, 1662 (1922).

TABLE XXVII

H <sub>2</sub> O <sub>2</sub> Taken (by Permanganate Titration), Milligrams	H <sub>2</sub> O <sub>2</sub> Found, Milligrams	Difference, Milligram
4.288	4.205	+0.083
1.860	1.829	+0.031
0.930	0.935	-0.005

A Duboscq colorimeter was used, the standard being set at 20.

## REFERENCES

1. Schön, *Z. anal. Chem.*, **9**, 41, 330 (1870).
2. Baerwald, *Ber.*, **17**, 1206 (1884).
3. Crismer, *Gaz. med. Liège*, **7**, 77 (1888).
4. Denigès, *Compt. rend.*, **110**, 1007 (1890).
5. Crismer, *Bull. soc. chim.*, [3] **6**, 22 (1891).
6. Nagel and Muthman, *Ber.*, **31**, 1836 (1898).

## DETERMINATION OF WATER IN "ABSOLUTE" ETHYL ALCOHOL \*

The method is based upon the fact that the sensitivity of azo indicators (e.g., methyl orange) to acid diminishes with increasing ethyl alcohol concentration of the medium. Designating the sensitivity quotient in water by 1, there is an increase to 135 in 91.5 per cent ethyl alcohol and then a decrease to 23 in 99.3 per cent ethyl alcohol. In making a determination, the colors of an aqueous and an alcoholic solution of the indicator are matched.

## Reagents.

1. Solution A. A saturated solution of methyl orange in strong ethyl alcohol.
2. Solution B. 0.01 N HCl in water.
3. Solution C. 0.1 N HCl in strong ethyl alcohol (from C<sub>2</sub>H<sub>5</sub>OH and HCl gas).

**Procedure.**—Place 25 cc. of water in a Nessler cylinder, add 0.1 cc. of solution A and 0.4 cc. of solution B. The intermediate orange-red color of methyl orange is obtained. In a second Nessler cylinder

\* I. M. Kolthoff, *Pharm. Weekblad*, **60**, 227 (1923).

place 25 cc. of the ethyl alcohol to be tested, add 0.12 cc. of solution *A*, and then solution *C* from a Bang burette until the color is the same as in the first cylinder. Note the temperature of the alcohol solution. For temperatures above 15° and concentrations between 99.7 and 95.0 per cent interpolations may be made from the following table prepared by Kolthoff: <sup>7</sup>

TABLE XXVIII

C <sub>2</sub> H <sub>5</sub> OH Volume, Per Cent	0.1 N Alcoholic HCl to Match Color of Aqueous Solution, Cubic Centimeters	Correction for Each Degree above 15
99.7	0.21	0.007
99.0	0.96	0.03
98.0	2.27	0.07
97.0	3.45	0.10
96.0	4.30	0.12
95.0	5.05	0.13

<sup>7</sup> *Loc. cit.*

## CHAPTER XXVIII

### PHOSPHORUS

#### DETERMINATION OF PHOSPHORUS AS PHOSPHOMOLYBDATE

THE method is based upon the yellow color of phosphomolybdate produced when nitric acid and ammonium molybdate are added to a solution containing phosphate. It is applicable to the determination of phosphates in soil and plant extracts and in natural waters. Silica interferes, owing to the formation of yellow silicomolybdate similar to phosphomolybdate and, hence, must be removed.

#### Reagents.

1. Nitric acid, sp. gr. 1.07.
2. Ammonium molybdate. Dissolve 50 grams of ammonium molybdate in a liter of water.
3. Standard phosphate solution. Dissolve 0.5043 gram of disodium hydrogen phosphate,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , in water, add 100 cc. of nitric acid, sp. gr. 1.07, dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of  $\text{P}_2\text{O}_5$ .

**Procedure.**—A measured sample containing less than 0.5 mg. of phosphorus is evaporated almost to dryness, 3 cc. of nitric acid added, and the residue heated for 2 hours at  $100^\circ \text{C}$ . to dehydrate the silica. (If the sample contains no silica, the dehydration is omitted.) The residue is taken up in water, transferred to a Nessler cylinder or colorimeter tube, 5 cc. of nitric acid, sp. gr. 1.07, and 4 cc. of ammonium molybdate solution are added and, after standing 20 minutes, the color is matched against a standard. Comparison may be made with a series of standards or by the balancing or dilution method. The series of standards must be prepared fresh daily, since the lighter solutions fade and the darker ones precipitate.

If the balancing or dilution method is used, 10 cc. of the standard phosphate solution are diluted with 75 cc. of water, 5 cc. of the ammonium molybdate solution added, the solution made up to 100 cc., and

thoroughly mixed. This solution contains 0.01 mg. of  $P_2O_5$  per cubic centimeter.

#### Notes.

1. The method of duplication is not convenient because of the time required for the full intensity of the color to develop.

2. Woodman and Cayvan<sup>1</sup> found that evaporation with nitric acid and heating at  $100^\circ$  for an hour was insufficient to render the silica entirely insoluble. When heated two hours at  $100^\circ$  no silica remained soluble. Heating for one hour at  $135^\circ$  did not render the silica completely insoluble.

3. It is not necessary to filter off the silica after dehydration. The loss due to filtration would be greater than any error due to the presence of a small precipitate of silica in the solution.

4. Iron salts affect the results if present in more than 20 parts per million. In aqueous extracts of soils a concentration greater than 0.1 to 5 parts per million is seldom obtained.

5. In case of doubt as to the purity of the sodium acid phosphate used in preparing the standard phosphate solution, the latter should be standardized by the magnesium ammonium phosphate method or the ammonium phosphomolybdate method. The concentration of the solution may then be adjusted to contain 0.1 mg. of  $P_2O_5$  per cubic centimeter.

#### DETERMINATION OF PHOSPHORUS BY SEPARATION AS MAGNESIUM AMMONIUM PHOSPHATE AND ESTIMATION OF THE PHOSPHATE AS PHOSPHOMOLYBDATE

This method is similar to the one used for estimating magnesium (p. 266). The phosphate is precipitated by magnesium chloride reagent in the form of magnesium ammonium phosphate and the phosphate in the latter determined by solution in dilute nitric acid and addition of ammonium molybdate which gives a yellow solution due to the formation of ammonium phosphomolybdate. This yellow solution is matched against a standard phosphomolybdate solution.

#### Reagents.

1. Nitric acid, sp. gr. 1.07.

2. Ammonium molybdate solution. Fifty grams of the pure salt are dissolved and the solution diluted to a liter.

<sup>1</sup> J. Am. Chem. Soc., **23**, 96 (1901).



3. Standard phosphate solution. Dissolve 0.5043 gram of pure freshly crystallized disodium phosphate,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , in water, add 100 cc. of nitric acid (sp. gr. 1.07), dilute to a liter, and thoroughly mix. One cubic centimeter of this solution is equivalent to 0.1 mg. of  $\text{P}_2\text{O}_5$  or 0.0342 mg. of Mg.

4. Standard colorimetric solution. Dilute 10 cc. of the standard phosphate solution (3) to about 80 cc., add 9 cc. of nitric acid (1) and 8 cc. of ammonium molybdate solution (2), and dilute to 100 cc. Mix thoroughly and allow to stand 20 minutes. One cubic centimeter of this solution is equivalent to 0.01 mg.  $\text{P}_2\text{O}_5$ .

5. Ammonium hydroxide, 6 N.

6. Ammonium hydroxide wash solution. Dilute 1 part of strong ammonia (sp. gr. 0.90) with 9 parts of water. The ammonia must be free from silica and, hence, only redistilled ammonium hydroxide should be used.

7. Ammonium oxalate. Saturated solution.

8. Magnesium chloride reagent. Thirteen grams of magnesium chloride,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , and 20 grams of ammonium chloride are dissolved in about 900 cc. of water, 50 cc. of strong ammonium hydroxide (sp. gr. 0.90) added, and the solution diluted to a liter. Thoroughly mix. One cubic centimeter of the solution will precipitate 3.5 mg. of  $\text{P}_2\text{O}_5$ .

9. Filter paper. Use only silica-free paper.

**Procedure.**—Measure out a sample which contains between 0.0005 and 0.004 gram of phosphorus and dissolve if a solid. Make solution faintly ammoniacal by adding 1 drop excess of 6 N ammonium hydroxide and add 2 or 3 drops of the ammonium oxalate solution. Evaporate to dryness on a water-bath, cool, and add 1 cc. of the magnesium chloride reagent. Thoroughly stir the precipitate and allow it to stand 2 or 3 hours. Then wash down the sides of the dish with 5 cc. of ammonium hydroxide wash solution and filter off, on a small filter, the magnesium ammonium phosphate. Repeat washing the dish with successive small amounts of the wash liquid until all the precipitate has been transferred to the filter, finally washing down the filter until the filtrate measures about 50 cc. Wash the dish once with about 5 cc. of cold water, allowing the water to run through the filter in such a way as to wash it. Reject the washings and place a small beaker under the funnel. Add 5 cc. of nitric acid to the evaporating dish, thoroughly spread it so as to insure complete removal of any precipitate that may

have remained on the sides of the dish, and then pour the solution through the filter in such a way as to wet the whole of it. Wash the dish four or five times with hot water (about 5 cc. each time) and continue washing the filter until the filtrate increases to about 45 cc. Cool the filtrate, add 4 cc. of the ammonium molybdate solution, dilute to 50 cc., mix, let stand 20 minutes, and compare the color with that of the standard phosphate solution, by the balancing or dilution method.

#### Notes.

1. The yellow color which develops is at its maximum intensity after 20 minutes and, hence, the solution must be allowed to stand this period before making the comparison. If the color is too strong for direct comparison with the standard, an aliquot part is used.

2. Great care must be taken to add enough of the molybdate reagents. The 5 cc. of nitric acid and 4 cc. of ammonium molybdate solution given in the procedure are sufficient only up to about 0.0003 gram of magnesium. When a second portion of these reagents is required (as indicated by the amount of precipitate or development of color), the solution is diluted with water at the same time so as to keep the concentration of the reagents the same, i.e., 5 cc.  $\text{HNO}_3$  and 4 cc. of molybdate solution per 50 cc. of the solution.

3. The 2 or 3 drops of ammonium oxalate solution are added before adding the phosphate reagent in order to prevent the calcium precipitating as calcium phosphate.

4. Silica gives a yellow color with the molybdate reagent, as does phosphate, and hence must be removed. In fact, the color produced by the silicomolybdates is even more intense than that of the phosphomolybdates.<sup>2</sup> Since an alkaline liquid is used throughout the procedure, dissolved silica will always be present and is removed in the rejected washings. The last washing must be made with pure water on account of traces of dissolved silica always present in ammonia water. Use only freshly distilled ammonia for preparing the wash solution.

5. If the sample is a solid, it is dissolved in the smallest amount of nitric or hydrochloric acid possible and the excess acid removed by evaporation to dryness. The residue is dissolved in water and the procedure continued in the usual way. Should the sample be a liquid,

<sup>2</sup> O. Schreiner and B. E. Brown, J. Am. Chem. Soc., **26**, 1463 (1904).

say, a potable water, it may be necessary to concentrate by evaporation. The phosphorus content of the sample should be between 0.0005 and 0.004 gram.

6. The standard phosphate solution is acidified with nitric acid in order to lessen contamination with silica from the glass bottle.

7. Any coloring matter in the sample is entirely removed or destroyed during the procedure and hence has no influence on the final color comparison.

8. Schreiner and Brown<sup>3</sup> report a list of 38 determinations which shows the method to be quite satisfactory. The following have been selected as representative of their results:

TABLE XXIX

Milligrams $P_2O_5$		Parts $P_2O_5$ per Million of Solution	
Present	Found	Present	Found
1.250	1.245	25.00	24.90
1.000	1.010	20.00	20.20
0.625	0.621	12.50	12.42
0.500	0.500	10.00	10.00
0.313	0.312	6.26	6.24
0.250	0.252	5.00	5.04
0.100	0.105	2.00	2.10
0.050	0.054	1.00	1.08

In order to determine the influence of various salts, especially silicates, Schreiner and Brown made 30 determinations with varying amounts of phosphates and constant amounts of the silicates, sulfates, chlorides, and nitrates of sodium, potassium, calcium, and magnesium. The approximate concentrations of the ions, other than phosphate, were as follows:

	P.p.m.		P.p.m.
Mg.....	10.0	$SO_4$ .....	40.0
Ca.....	10.0	Cl.....	20.0
K.....	10.0	$NO_3$ .....	15.0
Na.....	5.0	$SiO_2$ .....	10.0

Total salts = 120 p.p.m. of solution.

<sup>3</sup> *Loc. cit.*

The following six analyses are taken as representative of the 30 reported by Schreiner and Brown:

TABLE XXX

Milligrams $P_2O_5$		Parts $P_2O_5$ per Million of Solution	
Present	Found	Present	Found
1.250	1.215	25.00	24.30
1.000	1.005	20.00	20.10
0.500	0.504	10.00	10.08
0.250	0.252	5.00	5.04
0.125	0.155	2.50	3.10
0.050	0.072	1.00	1.44

#### DETERMINATION OF PHOSPHORUS BY PRECIPITATION AS PHOSPHOMOLYBDATE AND REDUCTION WITH HYDRAZINE SULFATE<sup>4</sup>

When ammonium phosphomolybdate is warmed with a solution of hydrazine sulfate the phosphomolybdate is reduced and a blue solution is obtained, the intensity of the color being proportional to the amount of phosphorus present.

##### Reagents.

1. Nitric acid, sp. gr. 1.125.
2. Ammonium nitrate, 30 per cent.
3. Ammonium molybdate, 3 per cent.
4. Hydrazine sulfate, 2 per cent.
5. Standard phosphate solution. Dissolve 0.5043 gram of pure crystallized disodium acid phosphate,  $Na_2HPO_4 \cdot 12H_2O$ , dilute to 100 cc. and thoroughly mix. One cubic centimeter of the solution contains 1 mg. of  $P_2O_5$ .

**Procedure.**—The sample solution containing 1 mg. or less of phosphate is concentrated to a volume of 1 or 2 cc. and transferred to a graduated centrifuge tube, ammonium nitrate solution being used to rinse the vessel. Add 1 cc. of nitric acid, sp. gr. 1.125, and more ammonium nitrate solution to bring the liquid in the tube up to 10 cc.; heat the tube to about 60°, add 2 cc. of ammonium molybdate solution,

<sup>4</sup> E. Riegler, Bull. Acad. Sci. Roumanie, **2**, 272 (1914).

thoroughly mix, and centrifuge for 2 or 3 minutes. Carefully pour off the clear liquid, add 5 cc. of ammonium nitrate solution, again centrifuge, and pour off the clear solution. Wash the precipitate into a 100 cc. volumetric flask with 20 cc. of the hydrazine sulfate solution and warm. Cool, dilute to the mark with water and thoroughly mix. Transfer the solution, or an aliquot part, to a colorimeter tube or Nessler cylinder, and compare the color against a standard solution prepared in the same manner. Treating 1 cc. of the standard phosphate solution as outlined for the sample and finally diluting to 100 cc. will give a solution containing 0.01 mg. of  $P_2O_5$  per cubic centimeter.

#### Notes.

1. The blue comparison solution is quite stable.
2. Losana<sup>5</sup> uses a hot solution of sodium thiosulfate to reduce ammonium phosphomolybdate. The resulting blue solution is matched against a standard solution similarly prepared.

#### DETERMINATION OF PHOSPHORUS AS PHOSPHO-VANADIO-MOLYBDATE<sup>6</sup>

The method is adapted to the determination of phosphorus in steel and cast iron and is based upon the formation of a yellow solution of phospho-vanadio-molybdate. The intensity of the color is proportional to the phosphorus content of the sample.

#### Reagents.

1. Nitric acid, sp. gr. 1.20, HCl-free.
2. Potassium permanganate. Dissolve 8 grams of the salt and dilute to a liter.
3. Hydrogen peroxide. Free from HCl and  $H_3PO_4$ . Add 40 grams of sodium peroxide, little by little, to a mixture of 100 cc. of nitric acid, sp. gr. 1.42, and 900 cc. of water, keeping the solution quite cool.
4. Ammonium vanadate. Dissolve 2.345 grams of  $(NH_4)_4VO_4$  in 500 cc. of hot water, add 20 cc. of  $HNO_3$  (sp. gr. 1.2), and dilute to a liter.

<sup>5</sup> Giorn. chim. ind. applicata, **4**, 60 (1922).

<sup>6</sup> G. Misson, Chem. Ztg., **32**, 633; Ann. chim. anal. chim. appl., **4**, 267 (1922); cf. R. Schröder, Stahl u. Eisen, **38**, 316 (1918).

5. Ammonium molybdate. 100 grams of the salt per liter. Use only a freshly prepared solution.

**Procedure.**—One gram of the steel or iron is placed in an Erlenmeyer flask marked at 80 cc. and dissolved in 20 cc. of nitric acid, sp. gr. 1.20. Boil the solution, add 10 cc. of the potassium permanganate solution, and again boil. Next add 10 cc. of hydrogen peroxide solution, shake until the manganese dioxide has dissolved, add 10 cc. of the vanadate solution, and boil to decompose the excess of hydrogen peroxide. Cool, dilute to about 60 cc., add 10 cc. of the ammonium molybdate solution, and dilute to the mark. Allow the solution to stand 2 or 3 minutes and then match its color against a series of standard solutions obtained by similar treatment of steel samples of known phosphorus content.

#### Notes.

1. The standard steel comparison solutions will last some time, but it is best to prepare fresh ones each week.
2. Estimations can be made to about 0.005 per cent phosphorus.

#### DETERMINATION OF PHOSPHORUS BY MOLYBDIC ACID-QUININE REAGENT<sup>7</sup>

This method is based upon the yellow coloration produced by adding a molybdic acid-quinine reagent to a solution containing a small quantity of phosphate.

#### Reagents.

1. Nitric acid, sp. gr. 1.12.
2. Molybdic acid-quinine reagent. Dissolve 1 gram of quinine sulfate in dilute nitric acid, add saturated barium hydroxide solution until the sulfate is completely precipitated, filter, and add the filtrate to a solution of 40 grams of ammonium molybdate in 500 cc. of nitric acid (sp. gr. 1.20). Dilute the solution to a liter and mix.
3. Standard phosphate solution. Dissolve 0.5043 gram of pure crystallized disodium acid phosphate,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , in water, add 100 cc. of nitric acid, dilute to a liter and thoroughly mix. Dilute 50 cc. of the solution to a liter and thoroughly mix. One cubic centimeter of the diluted solution contains 0.005 mg. of  $\text{P}_2\text{O}_5$ .

<sup>7</sup> A. Grégoire, Bull. soc. chim. Belg., **29**, 253 (1920).

**Procedure.**—A sample containing between 0.002 and 0.025 mg. of  $P_2O_5$  is placed in a 50 cc. Nessler tube and diluted to 45 cc. Add 2 cc. of nitric acid (sp. gr. 1.12), 2 cc. of the molybdic acid-quinine reagent, dilute to 50 cc., gently mix, and compare the color against a series of standards prepared along with the sample and under the same conditions.

**Note.**

Small amounts of silica do not interfere, but iron should be removed previously by means of "cupferron" (the ammonium salt of nitrosophenylhydroxylamine,  $C_6H_5 \cdot N \cdot NO \cdot ONH_4$ ). Cupferron may be obtained in the market or prepared according to Kasanof.<sup>8</sup>

**DETERMINATION OF PHOSPHORUS BY HYDROGEN SULFIDE<sup>9</sup>**

When hydrogen sulfide is passed into an alkaline molybdate solution a yellowish red color is produced which is proportional to the molybdenum. If the phosphorus in a substance be precipitated as phosphomolybdate, the latter dissolved in sodium hydroxide solution and the resulting solution saturated with hydrogen sulfide, the phosphorus may be estimated indirectly from the molybdenum determination.

The method is adapted to the estimation of phosphorus in steels and pig iron.

**Reagents.**

1. Nitric acid, 2 per cent.
2. Sodium hydroxide, 0.1 N. Dissolve 4 grams of sodium hydroxide and dilute to a liter.
3. Hydrogen sulfide. See p. 240.
4. Standard phosphomolybdate solution. Precipitate ammonium phosphomolybdate by adding a solution of ammonium molybdate to a solution of sodium phosphate. Wash the precipitate thoroughly with 2 per cent nitric acid and dry in an oven at  $120^\circ$ – $130^\circ$  C. Place 0.0307 gram of the dried precipitate in a 500 cc. volumetric flask, add from a burette just sufficient 0.1 N sodium hydroxide for solution, and then one-half this amount in excess. Dilute to the mark and thoroughly

<sup>8</sup> J. Ind. Eng. Chem., **12**, 799 (1920).

<sup>9</sup> T. E. Hewitt, J. Am. Chem. Soc., **27**, 121 (1905).

mix. One cubic centimeter of this solution contains 0.0010 mg. of phosphorus. (See Note 5.)

**Procedure.**—About 2 grams of the steel, pig iron, or other substance, are dissolved and the phosphorus precipitated as ammonium phosphomolybdate as in the usual volumetric method for the determination of phosphorus. Collect the precipitate on a small paper filter, wash with 2 per cent nitric acid, place the stem of the funnel in the neck of a 100 cc. volumetric flask, wet the filter thoroughly with a little hot water, add from a burette 0.1 N sodium hydroxide solution until the precipitate just dissolves, and then run in one-half this volume in excess. Dilute to the mark with water and thoroughly mix. Place an aliquot part in a 50 cc. Nessler tube, half fill the tube with water and pass in hydrogen sulfide at a moderate rate for 5 minutes. Next place the tube in a vessel of boiling water, let stand for 5 minutes, remove, fill to the mark, mix, and compare with a standard prepared by treating in the same way 10 cc. of the standard phosphomolybdate solution.

#### Notes.

1. If too small an amount of sodium hydroxide is used in dissolving the phosphomolybdate precipitate a blackish solution will be produced upon adding hydrogen sulfide. A reasonable excess of sodium hydroxide in the solution does not interfere with the analysis.

2. The solution must be thoroughly saturated with hydrogen sulfide, otherwise a light-colored solution will be obtained. Passing the hydrogen sulfide into the solution at a moderate rate for 5 minutes is sufficient.

3. The solution is not affected by air during treatment but gradually darkens on standing. After the solution has been saturated with hydrogen sulfide, it is heated for 5 minutes in boiling water in order to make the color stable for at least 2 hours.

4. Using Nessler tubes of 16 mm. bore and a depth of liquid of 24.5 cc., Hewitt<sup>10</sup> was able to detect a difference of 0.00089 mg. of phosphorus. The method gives results in excellent agreement with both volumetric and gravimetric methods.

5. Upon drying the ammonium phosphomolybdate precipitate, it approximates the formula  $(\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_3$ . This formula requires 1.65 per cent of P, whereas the precipitates actually obtained are likely to vary between 1.60 per cent and 1.64 per cent or even outside

<sup>10</sup> *Loc. cit.*



these limits. A. Tamm<sup>11</sup> found 1.64 per cent; F. Hundeshagen<sup>12</sup> found 1.62 per cent; E. Raben<sup>13</sup> found 1.64 per cent; Chesneau<sup>14</sup> found 1.60 per cent. The factor 1.63 per cent is the one most usually recommended.<sup>15</sup>

The maximum amount of ammonium phosphomolybdate which can be satisfactorily dried and weighed is about 0.4 gram.

#### DETERMINATION OF PHOSPHORUS IN URINE AND BLOOD BY THE BELL-DOISY-BRIGGS METHOD

Bell and Doisy<sup>16</sup> have employed the blue color produced by the reduction of phosphomolybdic acid by hydroquinone for the determination of phosphorus in urine and blood. The blue color is formed in an alkaline solution, but unfortunately has the disadvantage of fading fairly rapidly. In acid solution in the first stage of the determination a stable green is formed which is proportional to the phosphorus content, but this color was not used by Bell and Doisy on account of the observation that both urines and trichloroacetic acid blood filtrates sometimes give a turbidity upon the addition of the acid molybdate solution. Briggs<sup>17</sup> modified the procedure and obtained a perfectly clear green with acid molybdate and hydroquinone. The acid green color, however, is considerably less intense than the alkaline blue and, therefore, not so accurate with a low phosphorus content. This disadvantage is offset by the stability of the green color.

It was also observed by Briggs " that when a little sodium sulfite is added to an acid solution containing phosphate and molybdate that the subsequent addition of hydroquinone causes the formation of a blue instead of a green color and of an intensity considerably greater than the green. This color does not depend upon reduction of the molybdic acid by  $\text{SO}_2$  since sodium sulfite, hydroquinone, and acid molybdate solutions when mixed give no color. The use of these modifications gives a clear blue, non-fading color for comparison, the

<sup>11</sup> Chem. News, **49**, 208 (1884).

<sup>12</sup> Z. anal. Chem., **28**, 141 (1889).

<sup>13</sup> Z. anal. Chem., **47**, 546 (1908).

<sup>14</sup> Compt. rend., **146**, 758 (1908).

<sup>15</sup> Cf. H. A. Fales, Inorganic Quantitative Analysis, p. 215. The Century Company, New York, 1925.

<sup>16</sup> J. Biol. Chem., **44**, 55 (1920).

<sup>17</sup> J. Biol. Chem., **53**, 13 (1922).

proportionality of which is exact over a wide range. The intensity of the color allows the determination of phosphate in 1 cc. of plasma."

#### Reagents.

1. Trichloroacetic acid, 20 per cent.
2. Sodium sulfite solution, 20 per cent. Keep well stoppered or make up fresh.
3. Hydroquinone. Dissolve 0.5 gram of hydroquinone in 100 cc. of water and add a drop of concentrated sulfuric acid to retard oxidation.
4. Ammonium molybdate. Dissolve 25 grams of ammonium molybdate in 300 cc. of water. Dilute with 200 cc. of sulfuric acid (prepared by adding 75 cc. of sulfuric acid, sp. gr. 1.84, to 125 cc. of water).
5. Standard phosphate solution for urine. Dissolve 0.4388 gram of potassium dihydrogen phosphate,  $\text{KH}_2\text{PO}_4$ , dilute to a liter, and thoroughly mix. One cubic centimeter contains 0.1 mg. of phosphorus. Add a little chloroform as a preservative.
6. Standard phosphate solution for blood. Dilute 25 cc. of the urine phosphate standard to 200 cc., mix, and preserve with chloroform. Two cubic centimeters of this solution contain 0.025 mg. of phosphorus.

The following procedures are according to Briggs.<sup>18</sup>

**Procedure for Blood or Plasma.**—A measured volume of plasma is transferred to a small Erlenmeyer flask, diluted with 3 volumes of water and 1 volume of 20 per cent trichloroacetic acid. The flask is stoppered with the thumb, shaken vigorously for a few seconds, and after standing about 10 minutes, the contents are transferred to a dry ashless filter. The filter funnels rest in long Pyrex test tubes and are covered by watch-glasses to prevent loss by evaporation. For the determination, transfer 5 cc. of the filtrate, equivalent to 1 cc. of plasma, to a 10 cc. volumetric flask or a long test tube graduated at 15 cc. For the standard, transfer 2 cc. of the diluted phosphate solution, to a similar flask or tube. To each then add 2 cc. of the molybdate solution, 1 cc. of the sodium sulfite solution, and 1 cc. of the hydroquinone solution, and dilute with water to the mark. Allow them to stand about 30 minutes for color production and compare in the colorimeter.

<sup>18</sup> *Loc. cit.*

**Procedure for Urine.**—Take 1 to 5 cc. of acidified urine or an amount equivalent to about 0.5 mg. of P, in a 100 cc. volumetric flask. In a similar flask, take 5 cc. of the urine P standard. Dilute each with water up to about 80 cc. Then add to each 5 cc. of the molybdate solution, 1 cc. of the sulfite solution, and 1 cc. of the hydroquinone solution. Dilute each with water up to the mark and allow to stand about 30 minutes for color production.

#### Notes.

1. In the procedure for blood and plasma it is not necessary to add trichloroacetic acid to the standard to balance that of the filtrate. It is necessary, however, to have the acidity within certain limits for color production. Sufficient acid is provided by 2 cc. of the molybdate reagent for the formation of ammonium phosphomolybdate and its subsequent reduction; on the other hand if the total acidity after the addition of all reagents is more than 2 N then no color will be obtained.<sup>19</sup>

2. Denis<sup>20</sup> has shown that oxalates and citrates interfere with the formation of the alkaline blue color according to the Bell-Doisy method. However, when these salts are present in such amounts as used to prevent clotting of blood they do not interfere with the formation of the acid blue by the modified procedure of Briggs.

#### DETERMINATION OF PHOSPHORUS IN BLOOD AND URINE BY THE FISKE AND SUBBAROW METHOD<sup>21</sup>

This method is based upon the reaction between phosphate and ammonium molybdate to form ammonium phosphomolybdate and the reduction of the latter to a blue compound by aminonaphtholsulfonic acid. The resulting blue solution is then compared with a standard similarly prepared.

#### Reagents.

1. Sulfuric acid, 10 N. Add 450 cc. of sulfuric acid, sp. gr. 1.84, to 1300 cc. of water.

2. Trichloroacetic acid, 10 per cent. The quality of this reagent

<sup>19</sup> Briggs, *loc. cit.*

<sup>20</sup> J. Biol. Chem., **52**, 1 (1922).

<sup>21</sup> J. Biol. Chem., **66**, 375 (1925); see also Hawk and Bergeim, *Practical Physiological Chemistry*, 9th ed., pp. 403 and 776. P. Blakiston's Son and Co., Philadelphia, 1926.

is of great importance. The presence of certain impurities greatly retards the color development. Merck's U. S. P. product is free from such impurities, but contains a trace of phosphate. The amount of phosphate must either be determined in each sample or the acid purified by distillation. The phosphate may be determined as follows:

Arrange three tall beakers of 150 cc. capacity on a piece of white paper. Into one of these (*A*) put 100 cc. of water. In a second beaker (*B*) mix 85 cc. of water, 10 cc. of Molybdate I, and 4 cc. of 0.25 per cent aminonaphtholsulfonic acid; the result should be a solution practically as colorless as water, without a trace of blue (otherwise one or more of the reagents already added contains phosphate). To the third beaker (*C*) add 40 cc. of the trichloroacetic acid solution, 45 cc. of water, 10 cc. of Molybdate II, and 4 cc. of the sulfonic acid reagent, stirring thoroughly with a clean glass rod. Into *B* now run 1 cc. of a dilute phosphate solution containing 0.005 mg. of phosphorus per cubic centimeter, and mix well. Proceed in the same way, adding 1 cc. of this phosphate solution at intervals of not less than 2 minutes, until *B* and *C* appear to have the same color when examined from above. The volume of phosphate solution which must be added to bring this about, multiplied by 0.05, is the correction (in mg. per 100 cc.) to be subtracted from the result in the analysis of blood.

3. 1, 2, 4-Aminonaphtholsulfonic acid. This acid may be prepared from  $\beta$ -naphthol according to Folin's method,<sup>22</sup> with a single alteration. The final product, after washing with cold water, still contains some colored material. This is removed by washing the crystals, while still wet and on the filter, with alcohol as long as any color is extracted.

The reagent may also be obtained in satisfactory condition by one recrystallization of "technical" aminonaphtholsulfonic acid (Eastman Kodak Co., Rochester, N. Y.), as follows: Heat 1000 cc. of water to about 90° C., and dissolve in it 150 grams of sodium bisulfite and 10 grams of crystalline sodium sulfite. To this mixture add 15 grams of the crude sulfonic acid, and shake until all but the amorphous impurity has dissolved. Filter the hot solution through a large paper (about 32 cm.), cool the filtrate thoroughly under the tap, and add to it 10 cc. of concentrated hydrochloric acid. Filter with suction, wash with about 300 cc. of water, and finally with alcohol until the washings are colorless.

<sup>22</sup> J. Biol. Chem. **51**, 386 (1922).

The purified sulfonic acid should be dried in air with the least possible exposure to light, then powdered and transferred to a brown bottle.

4. Aminonaphtholsulfonic acid, 0.25 per cent. Dissolve 0.5 gram of the dry powder (obtained above) in 195 cc. of 15 per cent sodium bisulfite, add 5 cc. of 20 per cent sodium sulfite, stopper, and shake until dissolved. If the bisulfite solution is old, more than 5 cc. of sulfite will be needed, in which case add more sulfite, 1 cc. at a time with shaking after each addition, until solution is complete. This reagent can be prepared in a few minutes. Only an approximate weight of the powder need be made. If protected from the air the solution should keep about two weeks. It is more stable the higher the acidity, hence no more sulfite should be added than is required to dissolve the reducing agent.

5. Molybdate I, 2.5 per cent ammonium molybdate in 5 N sulfuric acid. Dissolve 25 grams of the salt in 200 cc. of water, rinse into a graduated liter flask containing 500 cc. of 10 N sulfuric acid, dilute to the mark with water and mix.

6. Molybdate II, 2.5 per cent ammonium molybdate in 3 N sulfuric acid. Prepare as above, but with only 300 cc. of 10 N sulfuric acid. This reagent is used only in the determination of inorganic phosphate in blood filtrates.

7. Molybdate III, 2.5 per cent ammonium molybdate in water. This solution must be discarded as soon as a considerable amount of sediment (ammonium trimolybdate) forms.

8. Sodium bisulfite. This solution must be free from turbidity before it can be used. In case the freshly prepared solution does not filter clear, let it stand 2 or 3 days before filtering. Keep well stoppered.

9. Sodium sulfite. Dissolve 200 grams of crystalline sodium sulfite,  $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ , in 380 cc. of water, filter if necessary, and keep stoppered.

10. Standard phosphate solution. Dissolve 0.3510 gram of pure monopotassium phosphate,  $\text{KH}_2\text{PO}_4$ , in water, transfer quantitatively to a graduated liter flask, add 10 cc. of 10 N sulfuric acid, dilute to the mark, and mix thoroughly. Five cubic centimeters of this solution contain 0.4 mg. of phosphorus. The standard keeps indefinitely.

**Procedure for Inorganic Phosphate in Blood.**—Transfer to an Erlenmeyer flask 4 volumes of 10 per cent trichloroacetic acid. While

the flask is being gently rotated, run in 1 volume of blood,<sup>23</sup> plasma, or serum—as the case may be—from a pipette calibrated for delivery (not contents). Close the mouth of the flask with a clean, dry rubber stopper, and shake vigorously a few times. The mixture may be filtered at once through an ashless paper.

Measure 5 cc. of the filtrate into a tube graduated at 10 cc. or a 10 cc. volumetric flask. Add 1 cc. of 2.5 per cent ammonium molybdate in 3 N sulfuric acid (Molybdate II), and finally (after mixing), 0.4 cc. of the usual sulfonic acid reagent. Dilute to the mark and mix. The standard, to be prepared as nearly as possible at the same time, is identical with the standard used for urine (0.4 mg. of phosphorus in a volume of 100 cc., or 0.2 mg. in a 50 cc. flask with half as much of each reagent), so blood and urine may be read against the same solution. It should be noted that the molybdate reagent added to the standard is always the one containing 5 N sulfuric acid (Molybdate I), and is different from that used for the blood filtrate. The purpose of this, as stated elsewhere, is to compensate for the high concentration of trichloroacetic acid in the filtrate.

The reading, as with urine, may be made in about 5 minutes, but it should be repeated a few minutes later if the color is particularly strong. To calculate the result in milligrams of phosphorus per 100 cc. of blood or other fluid (the standard being set at 20 mm.), divide 80 by the reading. From the figure so obtained subtract the correction for any phosphate which the trichloroacetic acid may contain.

If the inorganic phosphorus content is less than 2 mg. per cent, 1 cc. of the standard solution diluted 5 times (0.016 mg. P) should be added to the filtrate before adding the reagents or within 5 minutes after their addition.

**Determination of Total Acid-soluble Phosphorus in Blood.**—This determination is similar to the one above, except that the organic matter is destroyed by heating with nitric and sulfuric acids. Five cubic centimeters of the trichloroacetic acid filtrate should be used if possible. Boil this down, over a micro-burner, in a large lipped Pyrex test tube (200 by 25 mm.) with 5 cc. of 5 N sulfuric acid (or 2.5 cc. of 10 N) and a piece of quartz to prevent bumping. The bottom of the tube should be about 2 cm. above the burner tip. As soon as charring can be seen, or fumes appear, turn the flame down very low,

<sup>23</sup> Oxalate is the most suitable anticoagulant. Use 2, or at the most 3, mg. of potassium oxalate per cubic centimeter of blood.

so that the mixture barely boils, and heat until there is no further blackening. Now add 1 drop of nitric acid so that it runs down the wall of the test tube—it should not fall directly into the digestion mixture. If the color does not promptly disappear, add another drop of nitric acid in the same manner, and continue in this way until there is no color left. Nothing is gained by using a large amount of nitric acid. Ordinarily a single drop will be enough, and then about 30 seconds further boiling with the same low flame (to remove most of the nitric and nitrous acids left) will complete the ashing process.

Cool the tube under the tap, rinse the contents into a 50 cc. volumetric flask with 35 cc. of water, add 5 cc. of Molybdate III (2.5 per cent ammonium molybdate in water alone) and 2 cc. of the reducing agent. Dilute to the mark, and proceed as usual, reading against the standard that has been described before. 400 divided by the reading will give the desired result in milligrams per 100 cc. of blood.

The analysis may be made with 1 cc. of filtrate, using 1 cc. of 5 N sulfuric acid. The procedure is then otherwise the same as that described above, except that the final dilution must be 10 cc. (instead of 50) and the reagents diminished in proportion. It is safer, in this case, to use a smaller test tube (about 10 mm. in diameter) for the digestion. (See Note.) The most probable cause of loss of phosphate is superheating at the edge of the meniscus, which should consequently be as far removed as possible from the source of heat.

**Determination of Inorganic Phosphate in Urine.**—Measure into a 100 cc. volumetric flask enough urine to contain between 0.2 and 0.8 mg. of inorganic phosphorus (usually 1 or 2 cc.). Add water to bring the total volume to 70 cc., followed by 10 cc. of 2.5 per cent ammonium molybdate made up in 5 N sulfuric acid (Molybdate I), and 4 cc. of 0.25 per cent aminonaphtholsulfonic acid. After the addition of each reagent, the solution should be mixed by gentle shaking.

At the same time transfer to a similar flask 5 cc. of the standard phosphate solution (containing 0.4 mg. of phosphorus), 65 cc. of water, and the same reagents that were added to the urine sample. Dilute the contents of each flask to the mark, mix, and compare in the colorimeter after 5 minutes.

**Note.**—To prevent excessive foaming, some stable and not too volatile inhibiting agent (*e.g.*, phenyl ether) may be found helpful. It should be added from a tube drawn out to a fine capillary, so that

the drops are very small. Only 1 drop should be used to start with, and no more added later unless the first drop has all been driven off before the foaming stage is passed.

#### DETERMINATION OF INORGANIC PHOSPHATE IN BLOOD BY THE BENEDICT AND THEIS METHOD<sup>24</sup>

This method is the same in principle as that of Fiske and Subbarow (page 348). A solution of sodium bisulfite and hydroquinone is used as the reducing agent.

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Molybdate reagent. Add 25 cc. of 20 per cent sodium hydroxide solution to 20 grams of ammonia-free molybdic acid. Gently warm till solution is complete, cool, dilute to 250 cc. and filter if necessary.
3. Hydroquinone-sulfite solution. Dissolve 30 grams of sodium bisulfite in 100 cc. of water in a 200 cc. graduated flask, add 1 gram of hydroquinone, dilute to the mark, and mix.
4. Standard phosphate solution. Prepare a stock solution containing 0.4388 gram of  $\text{KH}_2\text{PO}_4$  per liter. Dilute 5 cc. of this solution to 100 cc. with water, thoroughly mix, and add a little chloroform to preserve. One cubic centimeter of the diluted solution contains 0.005 mg. of phosphorus.

**Procedure.** Place in a test tube 5 cc. of the filtrate obtained as in the Fiske and Subbarow method (page 348), and add 3 cc. of distilled water and 1 cc. of the molybdate reagent (diluted just before using with an equal volume of sulfuric acid, sp. gr. 1.84). Next add 1 cc. of the hydroquinone-sulfite solution, mix, stopper loosely, and place in a boiling water-bath for 10 minutes with a simultaneously prepared standard solution of potassium dihydrogen phosphate containing 0.025 mg. of phosphorus in 5 cc. which has been treated similarly to the blood filtrate.

##### Notes.

1. Using a standard containing 0.025 mg. of phosphorus, a satisfactory degree of accuracy is obtained with unknowns having a phosphorus content between 0.0125 and 0.05 mg.

<sup>24</sup> J. Biol. Chem., **61**, 63 (1924); see also Hawk and Bergeim, Practical Physiological Chemistry, 9th ed., p. 406. P. Blakiston's Son and Co., Philadelphia, 1926.



2. The color obtained by the above procedure is very intense and quite stable. The color of a standard solution was found to remain unchanged for several days. Heating longer than 10 minutes will produce a little increase in color intensity in both standard and blood filtrate, but the proportionality is the same at the end of an hour as at the end of 10 minutes.

**DETERMINATION OF PHOSPHATE AND SILICA IN THE PRESENCE  
OF EACH OTHER BY AMMONIUM MOLYBDATE**

For this determination see page 369.

## CHAPTER XXIX

### PLATINUM

#### DETERMINATION OF PLATINUM BY POTASSIUM IODIDE<sup>1</sup>

WHEN potassium iodide is added to a solution containing  $\text{PtCl}_6^{--}$  ions a red color develops, due to the formation of red  $\text{PtI}_6^{--}$  ions. The intensity of the color is proportional to the platinum content and hence may be made the basis for the determination of this element.

#### Reagents.

1. Hydrochloric acid, sp. gr. 1.19, and 1 N.
2. Nitric acid, sp. gr. 1.42, and 6 N.
3. Oxalic acid.
4. Potassium iodide, 2 per cent.
5. Zinc, 20-mesh.
6. Standard platinum solution. Dissolve a weighed quantity of pure platinum in aqua regia, evaporate to dryness, and dilute so that the solution contains 0.01 mg. of platinum per cubic centimeter.

**Procedure.**—Dissolve a convenient sized sample in hydrochloric acid and precipitate the platinum, gold, silver, and copper by treatment with 20-mesh zinc in hydrochloric acid. Filter, wash free from chloride, and dissolve the silver and copper in dilute nitric acid. Filter and thoroughly wash. Dissolve the platinum and gold in aqua regia, carefully evaporate to dryness, take up the residue in a little dilute hydrochloric acid, transfer the solution to a volumetric flask (100 or 250 cc.), and precipitate the gold with oxalic acid or ferrous sulfate. Dilute to the mark, thoroughly mix and allow the precipitate to settle. Pipette off an aliquot part of the clear liquid, transfer it to a Nessler tube, add 0.5 cc. of 1 N hydrochloric acid and 5 to 20 drops of 2 per cent potassium iodide solution. At the same time have ready a series of Nessler tubes containing measured quantities of the standard platinum solution and treat these in the same manner along with the

<sup>1</sup>E. G. R. Ardagh, F. S. Seaborne, and N. S. Grant, *Can. Chem. Met.*, 8, 117, 140 (1924).

sample. After the solutions have stood one hour at room temperature, the sample is matched against the standards.

**Notes.**

1. The optimum quantity of platinum is about 0.2 mg. per 50 cc. of solution.
2. The intensity of the color increases slowly on standing, about 90 per cent of the total intensity being reached in an hour. Comparison may be made at the end of one hour provided the potassium iodide solution was added to sample and standards at the same time, otherwise sufficient time must be given to insure maximum intensity.
3. The color does not develop as rapidly in freshly prepared solutions as in older ones, and this may be a grave source of error. The maximum intensity is, however, the same, regardless of the age of the solution.
4. The color matching is best made in a colorimeter but Nessler tubes are satisfactory.
5. It is advisable to allow the color to develop at room temperature.
6. All acids except hydrochloric are detrimental.
7. The quantity of potassium iodide used has little effect, provided sufficient is added to produce the full intensity of color.
8. The heavy metals likely to be present cause trouble, and hence must be removed.

## CHAPTER XXX

### POTASSIUM

#### DETERMINATION OF POTASSIUM BY PRECIPITATION WITH CHLORPLATINIC ACID AND REDUCTION WITH STANNOUS CHLORIDE<sup>1</sup>

THE potassium is precipitated as potassium chlorplatinate and the latter reduced with stannous chloride in the presence of hydrochloric acid. The yellow color produced by the stannous chloride is proportional to the amount of platinum present, which in turn is proportional to the potassium content. The standard solution is prepared from potassium chlorplatinate and, hence, the color comparison may be made directly in terms of the potassium.

#### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Hydrochloric acid, 6 N.
3. Chlorplatinic acid, 10 per cent.
4. Alcohol, 95 per cent.
5. Stannous chloride. About 100 grams of granulated or powdered tin are boiled in 500 cc. of concentrated hydrochloric acid in an Erlenmeyer flask until solution is about complete. The solution is kept in a tightly stoppered bottle containing a few pieces of mossy tin.
6. Standard potassium chlorplatinate. Dissolve 0.0516 gram of potassium chlorplatinate,  $K_2PtCl_6$ , in water and dilute to 1 liter. Mix thoroughly. One cubic centimeter of this solution contains 0.01 mg. of  $K_2O$ .

**Procedure.**—Measure out a sample of such size that its potassium content is between 0.1 and 1 mg. If a solid, dissolve in water, or hydrochloric or nitric acid if necessary. Add 1 cc. of sulfuric acid (sp. gr. 1.84), evaporate to dryness, and thoroughly ignite. Dissolve the residue in hot water, acidify with a few drops of hydrochloric acid, and add chlorplatinic acid in excess. Evaporate the solution to a thick

<sup>1</sup> L. A. Hill, J. Am. Chem. Soc., **25**, 990 (1903).

paste in a small dish, add a few cubic centimeters of alcohol, filter off the precipitate, and wash it thoroughly with alcohol, using successive small portions. Dissolve the precipitate in boiling water, cool, and dilute to 50 cc., or to 100 cc. if the potassium content is high. Transfer the solution (or one-half of it if diluted to 100 cc.) to a Nessler cylinder, add 3 cc. of the stannous chloride solution and mix. Compare the yellow color thus produced with that of a series of standard solutions prepared under similar conditions.

#### Notes.

1. The stannous chloride solution will produce a distinct yellow color when added to 50 cc. of a solution containing as little as 1 part of  $K_2O$  per million.

2. The following results were obtained by Hill.<sup>2</sup> They have been selected as representative of the 21 analyses reported.

TABLE XXXI

$K_2O$ Added p.p.m.	$K_2O$ Found p.p.m.
1.0	0.8
1.0	1.0
1.0	1.2
5.0	5.0
5.0	4.9
10.0	11.6
10.0	11.2

3. The method is especially suitable in the analysis of soil extracts, drainage waters, and also minerals and other substances in which the potassium content is too low to permit an accurate gravimetric determination.

4. If the color comparison is to be made by the balancing or dilution method, a suitable standard is obtained by adding 5 cc. of hydrochloric acid and 10 cc. of stannous chloride to 200 cc. of the standard potassium chlorplatinate solution and diluting to 250 cc. Thoroughly mix. One cubic centimeter of this solution contains 0.008 mg. of potassium.

<sup>2</sup> *Loc. cit.*

The method of duplication may also be employed. The standard potassium chlorplatinate solution is run into 25 cc. of water containing 3 cc. of stannous chloride and the final volume brought up to that of the sample by adding water.

#### DETERMINATION OF POTASSIUM BY THE ADDITION OF POTASSIUM IODIDE TO THE CHLORPLATINATE

This method<sup>3</sup> depends upon the formation of a pink color when potassium iodide is added to a dilute solution of potassium chlorplatinate containing one drop of concentrated hydrochloric acid. The most satisfactory range of concentration of potassium seems to be 1 to 10 parts per million. If a little alcohol is added with the potassium iodide and the solution heated, the pink color rapidly develops (always with a yellow or brown tinge) but changes in a few minutes into a clear yellow. Either the pink or the yellow color may be used for the comparison, the former being preferable for relatively low concentrations and the latter better for higher concentrations. (See Notes.)

##### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Sulfuric acid, sp. gr. 1.84.
3. Potassium iodide. Dissolve 86 grams of potassium iodide in water and dilute to a liter.
4. Alcohol, 95 per cent.
5. Standard potassium chlorplatinate solution. Dissolve 0.1032 gram of potassium chlorplatinate,  $K_2PtCl_6$ , in ammonia-free water, dilute to a liter, and thoroughly mix. For the series of standards or duplication method, 10 cc. of the standard solution are diluted to 100 cc. and mixed. One cubic centimeter of the solution contains 0.002 mg. of  $K_2O$ . If the method of dilution or balancing is to be used, 5 cc. of the standard solution are mixed with 5 cc. of potassium iodide solution and made up to 100 cc. This solution contains 0.001 mg. of  $K_2O$  per cubic centimeter.

**Procedure A: Pink Color Method.**—If the sample contains ammonium salts or organic matter these must be removed. This is accomplished by evaporating to dryness with a few drops of sulfuric

<sup>3</sup> F. K. Cameron and G. H. Failyer, *J. Am. Chem. Soc.*, **25**, 1063 (1903); *cf. Morrell, ibid.*, **2**, 145 (1880).

acid (sufficient to combine with all the bases) and then heating to a dull red heat, rotating the flame or dish so that all portions of the residue are heated thoroughly. Cool, add one drop of concentrated hydrochloric acid and then platinic chloride in slight excess. Rotate the dish so that the platinic chloride comes in contact with all portions of the residue, a few drops of ammonia-free water being added if necessary. Evaporate to dryness (or to a very stiff paste) on a water-bath, cool, and wash the residue on to an asbestos filter, using the smallest possible quantity of alcohol. Wash the precipitate six or eight times with alcohol, using 1 or 2 cc. portions and taking care to remove (by gentle suction) the alcohol as much as possible after each addition. After standing sufficiently long to insure the evaporation of all the alcohol, the precipitate is then washed through the asbestos filter with hot water. After the filtrate has cooled, add one drop of concentrated hydrochloric acid and then potassium iodide solution in large excess (at least 5 times the theoretical required to form  $K_2PtI_6$ ). Allow the solution to stand at least 4 hours in order that the maximum intensity of the pink color may develop. Then compare against a standard solution, diluting if necessary. Any of the usual methods of color matching may be employed, but Cameron and Failyer<sup>4</sup> used the method of balancing. (See Notes for some of their results.)

**Procedure B: Yellow Color Method.**—This method is carried out in the same way as Procedure A, except (1) that it is not necessary to remove by evaporation the alcohol used to wash the precipitate of chlorplatinate, and (2) that the addition of a drop of concentrated hydrochloric acid may be omitted, since the presence of free acid retards the development of the yellow color. To the hot water solution (without cooling) of the chlorplatinate add a large excess of potassium iodide solution (5 times the theoretical required to form  $K_2PtI_6$ ) and a small quantity of alcohol. Upon heating the solution a pink color, with a yellow or brown tinge, rapidly appears, and in a few minutes changes to a clear yellow. The solution is then matched against a standard prepared in a similar way.

If desired, the yellow color may be used as a check on the pink color. After the pink color has been compared with a standard, a little alcohol is added and the solution heated till the clear yellow color develops. On account of the free acid present in the pink solution, the development of the yellow color will be retarded.

<sup>4</sup> *Loc. cit.*

**Notes.**

1. The pink or rose color is probably due to the formation of a double salt of potassium iodide and platinic iodide or chloride. It is, of course, only an indirect method for the detection and estimation of potassium, the same reaction being used as a delicate test for small amounts of platinum.

2. Any ammonium salts present must be removed since they would precipitate a slightly soluble double chloride with platinic chloride. Organic matter would color the solution and hence must also be removed. If ammonium salts and organic matter are absent, the sulfuric acid treatment is omitted. One drop of concentrated hydrochloric acid and a slight excess of platinic chloride are added directly to the original solution and a single evaporation made.

3. All operations must be carried out so that no ammonia has access to the solutions. Use only ammonia-free water for reagents and for diluting.

4. An excess of platinic chloride must be used in order to precipitate all of the potassium, but all forms of platinum other than the potassium chlorplatinate must be removed before treating with potassium iodide. Potassium chlorplatinate is fairly soluble in alcohol but the rate of solution is so slow that the amount lost by rapid washing with small quantities of alcohol may be disregarded.

5. Experiments by Cameron and Failyer<sup>5</sup> have shown that five times the amount of potassium iodide theoretically required to form  $K_2PtI_6$  must be used. A larger excess showed no advantages.

6. The desirable tint of red is obtained in a strong solution and, hence, any necessary dilution is made only after the maximum pink color has developed. This requires about 4 hours or longer.

7. The one drop of concentrated hydrochloric acid is added to speed the formation of the pink color. Only a very small amount of free acid is permissible, since otherwise it would liberate iodine.

8. The yellow color produced by the presence of alcohol when potassium iodide is added to platinic chloride solutions is not as intense as the pink obtained when alcohol is absent, but is probably easier to "match" and is, therefore, recommended when relatively high concentrations are to be determined. It is also quite useful in that it affords a ready check upon the determination by the pink color.

<sup>5</sup> *Loc. cit.*



9. The pink color can be brought to its maximum intensity within a few minutes by warming the solution after adding the potassium iodide, but requires 4 hours or longer at room temperature. However, unless great care was taken to remove all the alcohol before dissolving the precipitate of potassium chlorplatinate, warming the solution will be likely to produce a brownish or yellowish tinge in the pink and, hence, make the comparison difficult, or even impossible. The alcohol may be removed by evaporation at room temperature. The evaporation may be hastened by drawing a slow current of air (preferably dry) through the precipitate.

10. Cameron and Failyer<sup>6</sup> have tested the accuracy of the "pink" method by using a series of solutions of potassium chlorplatinate. Their results are given in the following table in which potassium is expressed in parts per million of solution. A portion of (g) diluted four times was employed as a standard for the comparisons.

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>
Calculated.....	0.16	0.31	0.62	0.94	1.40	2.03	2.81
Found.....	0.17	0.33	0.64	0.94	1.40	2.01	2.81

The greatest divergence between the calculated and found amounts in this series is only one part in 50,000,000 parts of solution, although the percentage error is slightly greater than 6 per cent. Considering the magnitude of the quantities involved, the accuracy is excellent.

These authors also tested the accuracy of the method when other bases than potassium are present. The results they obtained while developing the method are given in the following table, as indicating the probable accuracy that may be expected when the method is being used by a person for the first time. With more experience, the accuracy of the method should be increased.

<sup>6</sup> *Loc. cit.*

TABLE XXXII

K Calculated P.p.m.	K Found P.p.m.	
50	52.7	50 parts Na, 50 parts Mg, and 50 parts Ca per 1,000,- 000 parts of solution, also present.
	48.0	
	48.0	
	53.5	
	52.7	
20	19.8	20 parts Na, 20 parts Mg, and 20 parts Ca per 1,000,- 000 parts of solution, also present.
	19.4	
	20.2	
	21.0	
	19.4	
5	6.4	5 parts Na, 5 parts Mg, and 5 parts Ca per 1,000,000 parts of solution, also present.
	6.4	
	6.9	
	6.0	
20	20.2	No other base present.
	20.1	
	20.1	
	20.0	
	12.7	Aqueous extract of a soil *
	13.8	
	30.6	Aqueous extract of a soil *
	30.2	
	58.0	Aqueous extract of a soil *
	61.0	

\* One of the eight extracts reported by C. and F. The data are representative.

11. Breazeale and Smith<sup>7</sup> have tested the above method independently, "against standards prepared by themselves, developing first the pink color and then subsequently developing the yellow color in the identical solutions which had just been read, and then re-reading." The test was made with a series of solutions containing known amounts of potassium and prepared by Cameron and Failyer. The following

<sup>7</sup> See Cameron and Failyer, *loc. cit.*

table gives some (about half) of their results selected as representative. The figures refer to parts of potassium per million of solution.

TABLE XXXIII

Found by Breazeale			Found by Smith	
Calculated	Pink	Yellow	Pink	Yellow
0.75	0.80	0.84	0.70	0.80
2.50	2.48	2.62	2.40	2.40
2.50	2.48	2.52	2.40	2.40
3.75	4.44	3.40	4.00	3.80
10.00	.....	10.00	.....	.....

A series of water extracts of soils containing other bases along with potassium was compared by Cameron and Failyer, using both the pink and the yellow color methods. Four of the eight extracts compared gave the following results, which are representative:

TABLE XXXIV

Pink K, p.p.m.	Yellow K, p.p.m.
2.4	2.8
5.7	5.4
17.7	17.1
23.9	26.2

## CHAPTER XXXI

### SELENIUM AND SILICON

#### DETERMINATION OF SELENIUM AS SELENIOUS ACID BY POTASSIUM IODIDE <sup>1</sup>

THE method is based upon the color produced by the action of selenious acid on potassium iodide.

##### Reagents.

1. Hydrochloric acid, 1.5 N.
2. Potassium iodide. Must be colorless.
3. Gum arabic solution.
4. Standard selenious acid solution. Dissolve 0.1632 gram of pure, crystalline selenious acid,  $\text{H}_2\text{SeO}_3$ , in water, dilute to a liter and mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of selenium. In case it is desired to report the results in terms of selenious acid, the standard is made by dissolving 0.1000 gram of  $\text{H}_2\text{SeO}_3$  per liter of solution. One cubic centimeter of this solution contains 0.1 mg.  $\text{H}_2\text{SeO}_3$ .

**Procedure.**—In case the sample is very concentrated in selenious acid, use 5 cc. A larger volume may be used for weaker solutions. A measured quantity of the sample is placed in a Nessler tube, one drop of gum arabic solution and 5 cc. of 1.5 N hydrochloric acid are added, the solution diluted to 99 cc., and mixed. One cubic centimeter of the potassium iodide solution is then added, the solution thoroughly mixed, allowed to stand 5 minutes, and then matched by dilution against 1 cc. of the standard treated in the same way as the sample.

**Note.**—The above method will detect as little as 0.001 mg. of selenium per cubic centimeter.

<sup>1</sup> J. Meyer and W. von Garn, *Z. anal. Chem.*, **53**, 29 (1914).

## DETERMINATION OF SILICON BY REDUCTION OF THE SILICOMOLYBDATE BY SODIUM SULFITE

## METHOD OF ISAACS

Silicates and phosphates form yellow silico- and phosphomolybdates with ammonium molybdate in acid solution. On treatment with sodium sulfite the silico- and phosphomolybdates give a blue reduction product. However, "silicomolybdates are reduced by sodium sulfite in the presence of a much lower concentration of hydrogen ions than is necessary for reduction of phosphomolybdates"<sup>2</sup> and it should, therefore, be possible to determine silicon, even in the presence of phosphates, by a suitable adjustment of the acidity before adding the sodium sulfite. Such a procedure has been developed by Isaacs<sup>3</sup> for the determination of silicon in tissues. The reliability of Isaacs' method has been confirmed by Foulger<sup>4</sup> who showed that (1) "phosphomolybdates do not give a blue reduction product when treated with sodium sulfite in the presence of acetic acid," and that (2) "quantitative mixtures of silicate and phosphate do not give a color *more* intense than would be given by solutions having the same concentration of silicate but no phosphate." The procedure given below is that of Isaacs.

## Reagents.

1. Nitric acid, sp. gr. 1.42.
2. Acetic acid, 10 per cent.
3. Boric acid. Use a saturated solution.
4. Sodium hydroxide, 2 per cent. Dissolve 1 gram of pure metallic sodium in 50 cc. of distilled water in a nickel vessel.
5. Ammonium molybdate, 10 per cent. This solution can be kept a week.
6. Sodium sulfite. Use a saturated solution.
7. Calcium nitrate,  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 5 per cent.
8. Standard silica solution. This solution can be prepared conveniently by dissolving a small amount of potassium silicate in a liter

<sup>2</sup> J. H. Foulger, J. Am. Chem. Soc., **49**, 434 (1927).

<sup>3</sup> Bull. soc. chim. biol., **6**, 157 (1924).

<sup>4</sup> *Loc. cit.*

of distilled water and then determining the exact concentration colorimetrically by comparison with a known weight of silica which has been fused with sodium carbonate and dissolved in a measured quantity of distilled water. The standard solution generally keeps 2 weeks, but occasionally a little silica precipitates after standing only a few days.

One cubic centimeter of the standard solution should contain 1 mg. of silica.

**Procedure.**—Weigh out 0.5 gram of dry tissue into a platinum crucible and add, successively, 1 cc. of boric acid solution, 1 cc. of calcium nitrate solution, and about 2 cc. of nitric acid. Place the crucible on the steam-bath until the tissue is dissolved; then heat it directly until the mass begins to char. Add more nitric acid and heat in such a manner as to obtain a white ash. The calcium nitrate facilitates this operation, and the boric acid prevents the loss of silicon as the tetrafluoride. The white ash is moistened with a few drops of nitric acid and heated gently until the excess acid is eliminated. The purpose of this operation is to convert the calcium oxide into the nitrate.

Next add 2 to 3 cc. of water and 3 cc. of sodium hydroxide solution. Heat to boiling and rotate the crucible so that the mass touches all sides. Add a sufficient quantity of acetic acid to neutralize the sodium hydroxide and then an excess of 3 cc., 10 cc. of water, and 5 cc. of ammonium molybdate solution. Transfer the solution to a Pyrex test tube graduated at 25 cc.

At the same time the sample solution is being prepared, a standard comparison solution is made by placing in a similar tube 1 cc. of the standard silica solution, 12 cc. of water, 3 cc. of acetic acid, and 5 cc. of ammonium molybdate solution.

The two tubes are put in boiling water for 5 minutes; then to each is added 2 cc. of sodium sulfite. A blue coloration develops. The colors are then compared in a colorimeter, or in Nessler cylinders by the method of dilution.

If the tissue contains blood, the iron changes the final color to green. In order to obtain a suitable standard for comparison, proceed as follows:

Place in a test tube 15 cc. of water, 3 cc. of 10 per cent acetic acid, 5 cc. of 10 per cent ammonium molybdate solution and 1 cc. of a 10 per cent solution of ferric ammonium alum. Place the tube in boiling water for 5 minutes and add 2 cc. of sodium sulfite solution. Thus a yellow solution is obtained which can be added to the comparison

standard so as to obtain a tint similar to that of the test solution. Strict account of the extent of dilution must, of course, be kept.

### Notes.

1. A correction must be made for silica in the reagents. Nitric acid, acetic acid, ammonium molybdate, sodium sulfite, and distilled water may be obtained silica-free. Boric acid and sodium hydroxide usually contain silica. This correction can be obtained by using a "blank." The nitric acid can be tested separately by adding 1 cc. of ammonium molybdate solution to 50 cc. of the acid. If no yellow coloration develops, the acid contains no silica.

2. Sometimes the test liquid is turbid. In such cases it should be filtered.

3. In more than one case Isaacs was able to determine as small a quantity of silicon as 0.5 mg. in 100 grams of dried tissue. Since he took only a 0.5 gram sample of tissue for ashing, his colored solutions in these cases contained only 0.0025 mg. of silica, or 0.00115 mg. of silicon. It is obvious that with such a sensitive method great care must be taken to avoid an error due to silica present in the reagents even in very small traces.

4. "Bertrand states that the color of the blue solution increases in intensity in course of time. This is true, but the fact does not invalidate Isaacs' method. If standard and test solution are prepared at the same time, even after standing for 27 hours, the ratio of color intensity of the two solutions is not greatly different from the color ratio of the freshly prepared solutions. With the standard at 10 mm. on the colorimeter scale in both cases, an unknown silicate solution matched the standard at 22.2 mm. when freshly prepared, and at 22.5 mm. 27 hours later. The calculated amounts of silicon in the unknown, on the basis of 0.7 mg. of silicon in the standard, were 0.315 and 0.311 mg., respectively. The difference corresponds to an error of 1.2 per cent, which is within the limits of error of a colorimetric method."<sup>5</sup>

5. "The reduction of silicomolybdates, in mixtures of silicomolybdates and phosphates, is retarded or inhibited by phosphates if the latter are present in sufficient concentration. Within the limits of phosphate concentration found in the ash from animal tissue, the

<sup>5</sup> J. H. Foulger, *loc. cit.*; cf. Bertrand, *Bull. soc. chim. biol.*, **6**, 656 (1924).

retarding action of phosphate can be removed by slightly increasing the acidity of the system before addition of the reducing agent.

"The method might be slightly improved, perhaps, in estimating silicon in the presence of large amounts of phosphorus by adding up to 7 cc. of 10 per cent acetic acid in making up the test solution. This still keeps the acidity far below that required for the reduction of phosphomolybdate."<sup>6</sup>

6. "The fact that both silicon and phosphorus can be estimated by the color produced on reduction of their molybdates would suggest that the results for the phosphorus content of tissues obtained by this method are actually estimations of phosphorus plus silicon. We have experimental evidence that some procedures for estimation of phosphorus by reduction of the phosphomolybdate can be used equally well for the estimation of silicon. This question is worthy of a separate investigation. We are undertaking further research on this point and on the whole mechanism of the reduction of molybdates."<sup>6</sup>

#### DETERMINATION OF SILICA AND PHOSPHATE IN THE PRESENCE OF EACH OTHER BY AMMONIUM MOLYBDATE

This method, due to Schreiner,<sup>7</sup> is based upon the yellow color of ammonium silicomolybdate and ammonium phosphomolybdate. Since silica solutions will give different intensities of color under different conditions, while phosphate solutions give the same depth of color under these different conditions, it is possible to estimate the two constituents simultaneously and in the presence of each other.

By experiment it has been shown that if ammonium molybdate and nitric acid are added to a silica solution an hour apart, the color of the silicomolybdate is half as great as the color produced by adding them simultaneously. Hence, by using two samples as follows, both silica and phosphate can be obtained. Add acid and molybdate to one and in 20 minutes take a reading [*A* reading]; to the other sample add molybdate only, let stand 1 hour and add acid, and in 20 minutes take a reading [*B* reading]. From these two readings the amounts of phosphate and silica can be calculated. If the phosphate content is less than the silica, as is frequently the case in natural waters, a mea-

<sup>6</sup> J. H. Foulger, *loc. cit.*

<sup>7</sup> O. Schreiner, *J. Am. Chem. Soc.*, **25**, 1056 (1903); *ibid.*, **26**, 808 (1904); A. T. Lincoln and P. Barker, *ibid.*, **26**, 975.



sured quantity of phosphate is added to equal or exceed the amount of silica present.

The method is applicable to the determination of phosphates and silica in soil and plant extracts and in waters.

### Reagents.

1. Nitric acid, sp. gr. 1.07.

2. Standard silica solution. Dissolve about 5 grams of precipitated and washed silica in an excess of sodium hydroxide made from metallic sodium. Make the solution faintly acid with nitric acid, dilute to a definite volume, mix, and determine the silica in an aliquot part. Dilute the strong solution until 1 cc. contains 1 mg. of  $\text{SiO}_2$ .

3. Standard phosphate solution. Dissolve 0.5043 gram of disodium hydrogen phosphate,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , in water, add 100 cc. of nitric acid, sp. gr. 1.07, dilute to a liter, and thoroughly mix. This solution contains 0.1 mg. of  $\text{P}_2\text{O}_5$  per cubic centimeter.

**Procedure**—Place two 50 cc. samples in Nessler tubes. To one add 5 cc. of nitric acid, sp. gr. 1.07, and 4 cc. of ammonium molybdate solution, let stand 20 minutes and compare with a standard phosphate solution prepared simultaneously by adding the same quantities of reagents. [*A* reading.] To the second sample add 4 cc. of ammonium molybdate solution, let stand 1 hour, then add 5 cc. of nitric acid, sp. gr. 1.07, and in 20 minutes compare with the standard. [*B* reading.] If phosphate is absent, the ratio of the *A* reading to the *B* reading is 2 : 1. If phosphate is present, the difference between the *A* reading and the *B* reading represents one-half the silica coloration, and twice this difference subtracted from the *A* reading gives the value of the color due to phosphate.

### Notes.

1. The 5 cc. of nitric acid, sp. gr. 1.07, added to 50 cc. of the sample has been found by experiment (Schreiner, and Lincoln and Barker)<sup>8</sup> to give the greatest intensity of color.

2. Schreiner<sup>9</sup> has pointed out that when the amount of phosphate is very low in comparison with the silica (as is frequently the case in drinking waters), a relatively considerable error in the phosphate

<sup>8</sup> *Loc. cit.*

<sup>9</sup> *Loc. cit.*

determination may result. It occurred to Lincoln and Barker<sup>10</sup> "that since Schreiner's method gives such good results when the  $P_2O_5$  and  $SiO_2$  are approximately equal or the  $P_2O_5$  in excess, *that if we were to add a quantity of  $P_2O_5$  sufficient to bring the  $P_2O_5$  content of the water up approximately to that of the  $SiO_2$ , or in excess of it, that the original amount of  $P_2O_5$ , and incidentally the  $SiO_2$ , could be readily and very accurately determined.*" This was tested thoroughly by Lincoln and Barker and found to be true. These authors examined between 5,000 and 6,000 water analyses by the Illinois State Water Survey in order to ascertain the maximum and minimum amounts of silica in the several types of water in the State of Illinois. They found that by adding 0.5 mg. of  $P_2O_5$  to 75 cc. of sample of water, very satisfactory results for  $P_2O_5$  and  $SiO_2$  can be obtained. By adding 0.5 mg. of  $P_2O_5$  to solutions of  $P_2O_5$  and  $SiO_2$ , Lincoln and Barker obtained the following results:

TABLE XXXV \*

Constituents Present, Milligram		Found, Milligram	
$P_2O_5$	$SiO_2$	$P_2O_5$	$SiO_2$
0.01	0.20	0.02	0.19
0.05	0.20	0.05	0.20
0.50	0.20	0.52	0.18
0.01	0.40	0.02	0.39
0.50	0.40	0.48	0.40

\* Only a few representative results are quoted here in order to save space.

3. Iron salts do not affect the results unless present to the extent of 20 or more parts per million of solution. In soil extracts, the concentration is seldom greater than 5 parts per million.

4. The different colorations produced by silica solutions under the two conditions in the above procedure are probably due to the formation of different silicomolybdates. Phosphate under these two conditions gives the same intensity of color and, hence, presumably the same phosphomolybdate.

<sup>10</sup> *Loc. cit.*

## CHAPTER XXXII

### SULFUR AND HYDROGEN SULFIDE

#### DETERMINATION OF SULFUR AS LEAD SULFIDE

THE method is used in the estimation of sulfur in iron, steel, etc. The metal is heated in a current of hydrogen with a mixture of sulfuric and hydrochloric acids, and the evolved gases are passed through a red-hot porcelain tube and finally absorbed in a potassium hydroxide solution of lead oxide. The precipitated lead sulfide is washed, dissolved in nitric acid, the solution neutralized, diluted, and ammonium sulfide added. The resulting colloidal solution of lead sulfide is matched against a standard prepared similarly.

#### Reagents.

1. Hydrochloric acid, 6 N.
2. Sulfuric acid, 6 N.
3. Nitric acid, 6 N.
4. Acetic acid, 6 N.
5. Sodium hydroxide, 6 N.
6. Lead oxide solution. Dissolve 10 grams of  $\text{PbO}$  in 250 cc. of strong potassium hydroxide solution.
7. Hydrogen. Purify the hydrogen by bubbling it through a copper sulfate solution.
8. Ammonium sulfide, 6 N. Use a freshly prepared solution.
9. Standard lead solution. Dissolve 51.65 grams of lead nitrate in water and dilute to a liter. Mix thoroughly. One cubic centimeter of this solution contains 32.31 mg. of lead and is equivalent to 5 mg. of sulfur.

**Procedure.**—Weigh out a sample sufficient to contain 0.1 to 10 mg. of sulfur, place in a vessel having an inlet- and an outlet-tube, the latter being connected to a porcelain tube heated red-hot and leading into a suitable absorption vessel containing a caustic potash solution of lead oxide. Add through a dropping funnel a mixture of dilute sul-

furic and hydrochloric acids. Hydrogen is then passed through the mixture. The evolved gases pass through the hot tube and then into the absorption vessel where lead is precipitated as the sulfide. Filter off the lead sulfide, wash it first with water and then with acetic acid, again wash with water, dissolve in nitric acid, neutralize the solution with sodium hydroxide, and dilute to a convenient volume. To the whole of the solution, or a suitable aliquot part, add 1 cc. of ammonium sulfide solution, mix gently, and match against a standard prepared by adding 1 cc. of ammonium sulfide solution to a measured quantity of the standard lead solution to which has been added a little sodium nitrate, corresponding to the amount present in the sample solution as the result of neutralizing the excess nitric acid.

#### Notes.

1. Since the color of the solutions is due to colloiddally divided lead sulfide, care must be taken to prepare the sample and standard under as near identical conditions as possible; otherwise the tints will not be the same, owing to a difference in the degree of dispersion of lead sulfide in each solution. Differences in electrolytes present have a marked influence on the color. Hence, the necessity of adding sodium nitrate to the standard in an amount corresponding to that in the final solution of the sample.

2. Color comparison should be made at once after the addition of the ammonium sulfide solution. The latter should be added to sample and standard at the same time.

#### REFERENCES

1. M. Lucas, *Bull. soc. chim.* [3], **17**, 150 (1897).
2. *Cf. ibid.* [3], **15**, 39 (1896) for Pb estimated as PbS.

#### DETERMINATION OF SULFUR BY THE METHYLENE BLUE METHOD<sup>1</sup>

The method is especially adapted to the estimation of sulfur in pig iron. The sulfur is evolved as hydrogen sulfide, the latter absorbed in an aqueous solution of sodium hydroxide, the solution diluted, and a portion of it acidified and treated with *p*-phenylenedimethyldiamine

<sup>1</sup>W. G. Lindsay, *Columbia University, School of Mines Quarterly*, **23**, 24; also in *Chem. Zentr.*, **1902**, i, 779; *cf.* F. D. Snell, *Colorimetric Analysis*, p. 135, D. Van Nostrand Co., New York, 1921.

hydrochloride and ferric chloride. The resulting blue color, due to methylene blue, is matched against a standard sodium sulfide solution similarly treated or against a standardized solution of methylene blue.

#### Reagents.

1. Hydrochloric acid, 6 N.
2. Sulfuric acid, 6 N.
3. Sodium hydroxide, 6 N.
4. *p*-Phenylenedimethyldiamine hydrochloride (unsymmetrical), 2 per cent.
5. Ferric chloride, 5 per cent.
6. Standard sulfide solution. Dissolve 0.2434 gram of sodium sulfide,  $\text{Na}_2\text{S}$ , in water, dilute to a liter, and mix thoroughly. To prepare a series of standards, dilute 5 cc. of the sulfide solution to 100 cc. The resulting solution contains 0.005 mg. of sulfur per cubic centimeter. Use from 1 to 25 cc. of this solution to prepare a series of varying sulfur concentrations. The diluted solution (0.005 mg. S per cubic centimeter) will also serve for the method of duplication. For the balancing or dilution method, treat 5 cc. of the first solution (0.2434 gram  $\text{Na}_2\text{S}$  per liter) with double the amounts of reagents used with the sample, dilute to 500 cc. and thoroughly mix. This solution contains 0.001 mg. of sulfur per cubic centimeter.

**Procedure.**—Two to 5 grams of the iron are treated with 6 N hydrochloric acid and the evolved hydrogen sulfide absorbed in an aqueous solution of sodium hydroxide, air being drawn by suction through the reaction flask and the absorption bulb. The alkaline sodium sulfide solution thus formed is diluted to 100 cc., mixed, and 10 cc. withdrawn as a sample. To the 10 cc. sample add 1.5 cc. of 6 N sulfuric acid, dilute to 50 cc., and add 0.1 cc. of the *p*-phenylenedimethyldiamine hydrochloride and 0.05 cc. of the ferric chloride solution. Mix thoroughly and compare the color by any of the usual methods.

#### Notes.

1. The standards are not very stable, and hence should be freshly prepared as needed.
2. *p*-Phenylenediamine, as well as various other diamines, may be used instead of the one above employed.

**DETERMINATION OF SULFUR BY THE ACTION OF HYDROGEN  
SULFIDE ON ARSENIOS OXIDE PAPER<sup>2</sup>**

The sulfur is liberated as hydrogen sulfide and the latter allowed to come in contact with arsenious oxide paper, thereby producing a yellow stain of arsenious sulfide. This stain is compared with a series of standard stains prepared along with the sample under similar conditions. Substances whose sulfur content is known are used in preparing the standard stains. Fresh stains must be made along with each sample or set of samples.

**Reagents.**

1. Hydrochloric acid, sp. gr. 1.1.
2. Benzine, sp. gr. 0.710.
3. Arsenious oxide paper. Make up a solution of 10 grams of arsenious oxide in 30 cc. of hydrochloric acid and 970 cc. of water. Soak in this solution strips of a good grade of drafting paper, remove the excess of reagent, dry the paper and cut it into squares 10 cm. on a side.

**Procedure.**—Place a weighed amount of the sample in a tall, round-rimmed beaker of suitable size; and in a series of similar beakers place weighed quantities of a steel (or substance similar to the sample) whose sulfur content is known. Completely cover each beaker with a square of arsenious oxide paper. Place a piece of felt upon each paper and a weight on top of each piece of felt. Now carefully add to each beaker 10 cc. of benzine and 50 cc. of the dilute hydrochloric acid, taking care to see that the paper is tightly held to the top of the beaker after the addition of the reagents. The hydrogen sulfide thus liberated comes in contact with the arsenious oxide on the paper and produces a yellow stain. The stain produced by the sample is matched against a series of standards simultaneously prepared along with the sample.

**DETERMINATION OF HYDROGEN SULFIDE BY FORMATION  
OF METHYLENE BLUE**

This method is based upon the action of ferric chloride and *p*-phenylenedimethyldiamine sulfate on an aqueous solution of hydrogen sulfide. Methylene blue is produced. It is especially adapted to the

<sup>2</sup> G. Misson, *Iron Age*, **93**, 1253 (1914); cf. F. D. Snell, *Colorimetric Analysis*, p. 136, D. Van Nostrand Co., New York, 1921.

estimation of hydrogen sulfide in natural waters and other solutions containing small quantities of hydrogen sulfide.

#### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Ferric chloride reagent. The solution is 0.1 N in  $\text{FeCl}_3$  and 6 N in HCl.
3. *p*-phenylenedimethyldiamine sulfate.
4. Standard hydrogen sulfide solution. Prepare a fresh solution of hydrogen sulfide as directed on page 240. Determine the hydrogen sulfide iodometrically. Measured volumes of this solution are then treated in the same way and along with the sample.

**Procedure.**—If the water contains between 1 and 3 mg. of hydrogen sulfide per liter, a sample of 500 cc. is taken and to it are added the following: 10 cc. hydrochloric acid (sp. gr. 1.19), 0.025 gram of *p*-phenylenedimethyldiamine sulfate, and 2.5 cc. of the ferric chloride reagent. If the water contains less than 1 mg. of hydrogen sulfide per liter, add the following to a 500 cc. sample: 10 cc. hydrochloric acid, (sp. gr. 1.19), 0.01 gram of *p*-phenylenedimethyldiamine sulfate, and 1 cc. of the ferric chloride reagent. Both the sample and standard having been treated similarly and simultaneously, they are allowed to stand several hours and the comparison of color then made by one of the usual methods.

#### Notes.

1. With solutions containing as little as 0.01 mg. of hydrogen sulfide per liter, vary satisfactory results may be obtained.<sup>3</sup>
2. Under the conditions given in the Procedure the intensity of the color developed is proportional to the amount of hydrogen sulfide present, and the color is stable for several weeks. Variations in the quantities, or order of addition of the reagents, alter the intensity of the color. The intensity of the color also varies with the temperature, being deeper the lower the temperature. Hence, the sample and standard must be treated under the same conditions and the directions of the Procedure followed as closely as possible. The temperature of the sample and standard should not differ by more than 2° C.

<sup>3</sup> W. Mecklenburg and F. Rosenkränzer, Z. anorg. Chem., **86**, 143 (1914).

## CHAPTER XXXIII

### TITANIUM

#### DETERMINATION OF TITANIUM IN IRON AND STEEL

##### METHOD OF McCABE<sup>1</sup>

THIS method is based upon Weller's hydrogen peroxide method.<sup>2</sup> In iron and steel analysis the color of the ferric sulfate in the sulfuric acid solution is usually much more intense than that of the titanium. The comparison color is therefore a blend of two colors, the titanium producing the subordinate one. If the same weight of iron is present in both standard and sample, it follows that *at equal volumes the color due to iron will be identical in the two solutions*. It is then only necessary to imitate the color of the sample solution by adding a measured quantity of a standard titanium solution to a solution of a non-titanium steel, adjusting both solutions to the same volume before the final comparison.

##### Reagents.

1. Sulfuric acid, 9 N. Add 1 volume of sulfuric acid, sp. gr. 1.84, to 3 volumes of water.
2. Nitric acid, sp. gr. 1.2.
3. Hydrochloric acid, sp. gr. 1.19.
4. Ether, alcohol-free.
5. Hydrogen peroxide, 3 per cent. Determine the approximate strength of the peroxide by titration against a standard permanganate solution. This should be done every few weeks, otherwise serious error may arise due to deterioration of the peroxide.
6. Standard titanium solution. Place about 100 grams of ferro-titanium in a dish and pour over it about 50 cc. of strong HCl. Heat,

<sup>1</sup> J. Ind. Eng. Chem., **5**, 735 (1913); see Note, *ibid.*, **5**, 872; Chem. Eng., **13**, 243 (1911), McCabe's original method.

<sup>2</sup> Ber., **15**, 2593 (1882); see also Schönn, Z. anal. Chem., **9**, 41, 330 (1870).



and when the solvent action has progressed for a few moments add 5 cc. of nitric acid (sp. gr. 1.2). Continue heating until the acid is about half evaporated, dilute with 15 or 20 cc. water and filter through a large paper filter. Treat the ferro-titanium again with hydrochloric and nitric acids, dilute, and filter in this manner a dozen times or more until the desired quantity of ferro-titanium has been dissolved. Simultaneously with the solution of the ferro-titanium the combined filtrates are evaporated in a large beaker on a hot-plate. Evaporate until separation of titanic acid is observed. Pour into several separatory funnels, and add to each 50 cc. of ether. Make repeated other extractions of the iron until the titanic acid is free of iron as shown by the potassium sulfocyanate test. Combine the several acid solutions and place in a 400 cc. beaker. To the titanic acid emulsion add 150 cc. sulfuric acid (1 : 3). Heat and filter from the insoluble portion, which is usually slight. Dilute the filtrate to about 700 cc. in a liter beaker, add ammonia in excess, boil, and allow the precipitate to settle. Decant or siphon away the supernatant solution and wash the hydrated titanic acid free of chlorides by decantation.

Dissolve the titanic acid in 50 cc. of sulfuric acid (1 : 1) and dilute to a liter. Determine the strength of this stock solution by precipitation of 100 cc. with ammonia. Calculate the dilution required to yield a standard solution containing 0.0002 gram of titanium per cubic centimeter. Remove the requisite quantity of the stock solution and dilute according to the calculation. Finally check the strength of the standard solution by precipitation of 100 cc. with ammonia.

The following procedures are according to McCabe.<sup>3</sup>

**Procedure A: When Titanium is above 0.02 Per Cent.**—Put 2 grams each of the titanium steel and a non-titanium steel into 300 cc. Erlenmeyer flasks. Pour into each flask 80 cc. of sulfuric acid (1 : 3) and heat on a hot-plate to complete solution. To each add 4 cc. of nitric acid (sp. gr. 1.2) and continue boiling until the solutions are free of fumes; cool. Transfer the solution of non-titanium steel to a comparing tube, using as little wash water as possible. Pour the test sample into the companion tube and dilute to the same volume as the other. Now observe if the two solutions have colors of the same character and depth, as they should. If the test sample is cast iron, filter the solution into the tube, preferably using a small wad of

<sup>3</sup> *Loc. cit.*

absorbent cotton because of gelatinous silica. Match the color with that of the non-titanium steel solution. Owing to the fact that cast iron contains a much larger percentage of metalloids than steel, it is usually found that the volume of the iron solution is a trifle less than that of the non-titanium steel when the colors match. In this case discard sufficient of the non-titanium steel solution to make the volumes equal.

Having obtained solutions showing the same color in equal volumes, introduce into each 2 cc. of 3 per cent hydrogen peroxide. Mix, and observe closely if the test solution shows any deepening of color. If titanium is present in as small an amount as 0.02 per cent, this deepening will be in evidence. It may even be detected with 0.01 per cent present, but in that case it is better to rely on the more refined method to be described.

Titanium, if shown to be present in notable quantity, is determined by adding from a burette a sufficient amount of standard titanium solution to the solution of non-titanium steel to imitate the color of the test solution. If but 2 or 3 cc. are required, the amount used may be accepted as representing the amount in the test solution without further concern. But, if more is required, the equality of volume of the two solutions, so vital to accurate results, must be restored before final comparison. When colors match at equal volumes, the amount of standard titanium solution used indicates the amount of titanium in the test sample.

**Procedure B: When Titanium is below 0.02 Per Cent.**—When the titanium content is less than 0.02 per cent and a high degree of accuracy is desired, McCabe's more refined method may be used. This requires the separation of the iron by careful extraction with ether.

Weigh 2 grams of a non-titanium steel and the same quantity of the test sample. Place in dishes, add 50 cc. strong hydrochloric acid and heat to complete solution. Add to each 4 cc. nitric acid (sp. gr. 1.2) to oxidize iron. Evaporate each solution to about 10 cc., cool the concentrated ferric chloride solutions, pour into separatory funnels, and wash with hydrochloric acid (2 parts strong acid : 1 part water) until the volume amounts to 25 cc. Add to each 50 cc. alcohol-free ether, agitate thoroughly, and allow to stand for 5 minutes after the two solutions have separated. Draw off the acid solution, avoiding the ether solution entirely even though a slight loss of acid solution may be necessary to do so.

Dilute each solution with 225 cc. hot water, add ammonia in slight excess, and boil. Allow the precipitates to settle and filter through 11 cm. filter papers. Wash thoroughly with hot water to remove chlorides.

Open the two filter papers into dishes, tearing off that portion of the filter paper which holds no precipitate. Pour over each 10 cc. of sulfuric acid (1 : 3). Rock the dishes until all the precipitate has dissolved. Filter each into 30 cc. comparison tubes, using small funnels and 7 cm. filter papers. This filtration may be dispensed with, but it is best to remove paper fiber. Wash the dishes and pass the washings through the filters. The total solution and washings in each instance should be equal in volume, between 15 and 18 cc.

The solutions now have a slight maroon color, depending on the amount of iron present. Into each tube introduce 3 cc. of 3 per cent hydrogen peroxide and mix. The maroon color is discharged, leaving the solution practically colorless in the case of the non-titanium steel. But if the test solution contains the most minute quantity of titanium, its presence is revealed by a residual lemon-yellow color. Even though the amount present is so extremely small that the usual manner of comparison leaves the operator uncertain of its presence, a glance through the solution from the top, holding the tube about  $\frac{1}{2}$  inch above a white surface, instantly dispels all doubt. If there is as little as 0.001 per cent titanium present, the contrast between the pale green of the non-titanium solution and the yellowish tinge of the test clearly indicates that fact.

By adding the standard titanium solution to the solution of non-titanium steel the percentage of titanium is readily determined.

#### Notes.

1. When cast iron is to be analyzed, it should be screened through a 60-mesh sieve. In dissolving the sample, the insoluble residue retains a small amount of titanium. For ordinary technical purposes this may be disregarded, but if an accurate analysis is required, or if the titanium content is small, the insoluble is ignited in a platinum crucible; the silica volatilized with hydrofluoric acid and sulfuric acid (1 : 1), the residue fused with 1 gram of sodium carbonate, dissolved in dilute sulfuric acid (in case of Procedure A) or in hydrochloric acid (in case of Procedure B), and added to the main solution.

2. The purpose of the ether extraction in Procedure B is two-fold:

- (1) to obtain the minimum quantity of iron in the acid solutions and
- (2) to have the iron content nearly the same in each solution.

#### DETERMINATION OF TITANIUM BY THYMOL

Thymol and titanium in concentrated acid solution give a reddish yellow to deep red coloration, the intensity of the color being proportional to the amount of titanium present. The method is applicable to smaller amounts of titanium than can be determined by the hydrogen peroxide method. "The intensity of the coloration produced by thymol in sulfuric acid solution with titanium is at least twenty-five times as great as that produced in the hydrogen peroxide method."<sup>4</sup>

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Potassium bisulfate. Pure solid.
3. Thymol reagent. Dissolve 5 grams of thymol in 5 cc. of dilute acetic acid, add 95 cc. of concentrated sulfuric acid and mix. Do not expose to direct sunlight.
4. Standard titanium solution. This solution may be prepared from pure titanium dioxide or from a standard ferro-titanium steel. Dissolve 0.1 gram of pure titanium dioxide (or an equivalent amount of ferro-titanium steel) in a little sulfuric acid and dilute to a liter with concentrated sulfuric acid. Thoroughly mix. One cubic centimeter of this solution contains 0.1 mg. of  $\text{TiO}_2$ . A measured volume of this solution may be further diluted with concentrated sulfuric acid, if a weaker standard is needed.

**Procedure.**—A sample containing 1 to 10 mg.  $\text{TiO}_2$  is accurately weighed out, dissolved directly in concentrated sulfuric acid, or, if necessary, fused with potassium acid sulfate for a half hour and the fused mass taken up in concentrated sulfuric acid. Transfer to a Nessler tube, add thymol reagent (at least 60 mg. of thymol to 1 mg. of  $\text{TiO}_2$ ) and dilute to the mark with concentrated sulfuric acid. Add the same *relative* quantity of thymol to a measured volume of the standard titanium solution. Match the color of the sample against that of the standard by the balancing method. Both solutions must be at room temperature. If desired, a series of standards containing various amounts of  $\text{TiO}_2$  may be prepared. If the dilution method is

<sup>4</sup>V. Lenher and W. G. Crawford, Orig. Com. 8th Intern. Congr. Appl. Chem., **1**, 285 (1912). Also in J. Am. Chem. Soc., **35**, 138 (1913).

used, the dilution must be made with sulfuric acid of at least 80 per cent strength.

### Notes.

1. "The ratio of thymol to titanium can vary greatly, but it has been found best to have at least 0.006 gram of thymol present to every 0.0001 gram of  $\text{TiO}_2$ ."<sup>5</sup>

2. The sulfuric acid concentration in both the sample solution and the standard must be at least 79.4 per cent (sp. gr. 1.725), otherwise the color obtained with thymol reagent will be paler than the normal intensity. (Lenher and Crawford.)

3. All color comparisons must be made with solutions at room temperature. Heating titanium solutions colored by thymol causes a fading in color, but the color returns to the original intensity upon cooling to 20° C., provided the temperature did not reach 100° C. (Lenher and Crawford.)

4. Hydrofluoric acid and fluorides bleach the color of titanium and thymol in concentrated sulfuric acid solution. Fortunately, from the preliminary treatment of the sample in order to bring it into concentrated sulfuric acid solution, it is practically impossible for fluorides to be present.

5. Chlorides, phosphates, and tin apparently have no effect on the thymol-titanium coloration. Tungstic acid, however, "markedly affects the color in direct ratio to the amount of tungsten present." (Lenher and Crawford.)

6. Lenher and Crawford<sup>6</sup> obtained the following results (representative) with the thymol method:

(a) Titanium Solutions compared in a Soleil-Duboscq Colorimeter.

$\text{TiO}_2$ Present, Milligram	$\text{TiO}_2$ Found, Milligram
0.21	0.20
0.21	0.19
0.50	0.50
0.50	0.52

<sup>5</sup> Lenher and Crawford, *loc. cit.*

<sup>6</sup> *Loc. cit.*

(b) Titanium Solutions compared in a Kennicott-Sargent Colorimeter.

TiO <sub>2</sub> Present, Milligrams	TiO <sub>2</sub> Found, Milligrams
1.0	0.9
2.1	2.0
2.5	2.5

(c) Four samples of bauxite previously analyzed and the titanium determined by Weller's hydrogen peroxide method.

"Samples of 0.3 gram each were fused with potassium bisulfate for a half hour, after which the fusion was taken up in concentrated sulfuric acid," thymol added, and the colors matched against a standard.

Weller's Method, Per Cent TiO <sub>2</sub>	Thymol Method, Per Cent TiO <sub>2</sub>	
	(1)	(2)
3.3	3.7	3.4
1.93	2.2	2.1
2.20	2.15	2.28
2.97	2.83	2.95

7. Hall and Smith,<sup>7</sup> and Lenher and Crawford<sup>8</sup> have found over fifty organic compounds which produce distinctive color reactions with titanium in concentrated sulfuric acid solution. Of these, thymol, phenol, hydroquinone, salicylic acid, and chromotropic acid are the most sensitive. The latter two indicate as little as 0.01 mg. TiO<sub>2</sub>, hydroquinone indicates 0.1 mg., and phenol 0.05 mg. However, for various reasons thymol produces the most satisfactory coloration for the determination of small amounts of titanium.

<sup>7</sup> R. D. Hall and E. F. Smith, Proc. Am. Phil. Soc., **44**, 196 (1905).

<sup>8</sup> *Loc. cit.*

**DETERMINATION OF TITANIUM BY HYDROGEN PEROXIDE  
(SODIUM PEROXIDE FUSION)**

The use of the yellow color produced when hydrogen peroxide is added to a sulfuric acid solution of titanium as a colorimetric method for low percentages of titanium was first proposed by Weller.<sup>9</sup>

This method has been extensively used in the analysis of clays, silicate rocks, and steels. Moreover, since the color is so easily bleached by fluorides, the latter action has been adapted to the quantitative estimation of fluorides.<sup>10</sup> (See page 186.)

**Reagents.**

1. Sulfuric acid, sp. gr. 1.4.
2. Phosphoric acid, 50 per cent.
3. Sodium peroxide.
4. Standard titanium solution. Fuse pure titanium dioxide with sodium peroxide, dissolve the fused mass in water, and add sufficient sulfuric acid for the solution to contain 5 per cent acid when diluted to the desired titanium concentration. If phosphoric acid is added to the solution of sample to repress the yellow color due to ferric iron, the same amount must be added to the standard. (See Note 1.)

**Procedure.**—If the clay or ore contains between 1 and 5 per cent of  $\text{TiO}_2$ , about 1 gram of the material is accurately weighed out, mixed thoroughly with 8 grams of sodium peroxide in an iron crucible, and heated until fusion is complete. The fused mass is then cooled, dissolved in 200 cc. of water and, without filtering, 15 cc. of sulfuric acid (sp. gr. 1.4) and 6 cc. of phosphoric acid (50 per cent) are added and the solution diluted to 250 cc. The color is then matched against a standard solution which contains the same amount of phosphoric acid as was added to the sample, i.e., 6 cc. of the 50 per cent acid per 250 cc. of solution.

**Notes.**

1. Walton<sup>11</sup> has shown that free phosphoric acid or phosphates diminish the depth of the yellow color of the titanium solution. Hence, the results will be low unless the same amount of phosphoric acid is

<sup>9</sup> Ber., **15**, 2593 (1882). See also Schönn, Z. anal. Chem., **9**, 41, 330 (1870).

<sup>10</sup> Steiger, J. Am. Chem. Soc., **30**, 219 (1908).

<sup>11</sup> J. Am. Chem. Soc., **29**, 481 (1907).

added to standard as was added to the solution of the sample. With substances containing a large amount of iron, the quantity of phosphoric acid should be increased.

2. The fusion of clays, ores, and glazes with sodium peroxide is rapid and complete. The fusion may be made in iron, nickel, or silver crucibles. Porcelain crucibles must not be used, because they often contain a small amount of titanium. About 0.2 gram of iron is added to the sample when the fusion is carried out in an iron crucible due to the action of the sodium peroxide on the crucible.

3. The presence of sodium peroxide from the fusion makes it unnecessary to add hydrogen peroxide, since the latter is formed upon acidifying the solution of the melt.

4. The yellow color of the peroxide solution of titanium fades upon standing. This is probably due to the ferric sulfate present. Walton observed "that in the presence of ferric salts the yellow color of the titanium disappears much more rapidly than in titanium solutions which contain no iron."

5. Using the method given in the above Procedure, Walton<sup>12</sup> obtained the following results with the several substances indicated.

TABLE XXXVI

Substances Analyzed	Per Cent Fe Present	Per Cent $\text{TiO}_2$ Obtained by Fusion with Acid Sulfate	Per cent $\text{TiO}_2$ Obtained by Fusion with $\text{Na}_2\text{O}_2$ and Addition of $\text{H}_3\text{PO}_4$ to Both Solution and Standard
Clay.....	4.3	1.13	1.13
Fireclay.....	2.7	1.30	1.26
Limestone.....	1.2	0.22	0.23
Iron Ore.....	30.0	3.73	3.66

6. Fluorides or hydrofluoric acid present in very small amounts cause considerable error due to their bleaching action on peroxide-titanium solutions. Walton<sup>12</sup> observed an error of 6.7 per cent in the colorimeter reading with a solution containing 0.010 gram  $\text{TiO}_2$  and 0.00039 gram HF. When the HF was increased to 0.0039 gram the error was 32.4 per cent.

<sup>12</sup> *Loc. cit.*



**METHYL ORANGE AS A PERMANENT STANDARD FOR THE DETERMINATION OF TITANIUM BY THE PEROXIDE METHOD**

Gautier<sup>13</sup> recommends the use of methyl orange for the preparation of a standard comparison scale in the colorimetric determination of titanium by the hydrogen peroxide method. The solutions do not fade and are easily prepared.

Dissolve 1 gram of methyl orange in 500 cc. Ten cubic centimeters of this solution are diluted to 200 cc. Using small volumes of the diluted solution, a series is made in which the color intensity varies over the desired range. Standardize against a standard titanium solution which has been oxidized by hydrogen peroxide. Keep solutions stoppered when not in use, to prevent evaporation.

<sup>13</sup> *Chimiste*, **2**, 2; *Rev. gén. Chim.*, **14**, 16 (1911).

## CHAPTER XXXIV

### TUNGSTEN AND VANADIUM

#### DETERMINATION OF TUNGSTEN AS THE OXIDE IN COLLOIDAL SUSPENSION <sup>1</sup>

THE method is based upon the reduction of  $\text{H}_2\text{WO}_4$  by  $\text{TiCl}_3$ , giving a blue oxide of tungsten that remains in colloidal suspension under certain conditions.

##### Reagents.

1. Hydrochloric acid, 1 N and 6 N.
2. Ammonium hydroxide, 6 N.
3. Titanium chloride solution. (Should correspond to 2 mg. Fe per cubic centimeter.)
4. Sodium sulfite, solid, anhydrous.
5. Standard tungsten solution. Weigh out a sample of tungsten steel whose tungsten content is known, using a weight that contains approximately the amount of tungsten estimated to be in the sample. Then add a non-tungsten steel until the total weight is equal to the weight of sample taken. Dissolve the standard and treat by the same method as used for the sample.

If the sample is a mineral, use a high-tungsten steel and omit adding a non-tungsten steel.

**Procedure.**—A sample is taken such that the final comparison solution will not contain over 1 mg. of tungsten per cubic centimeter. When the tungsten content is very high, an aliquot part of the solution of the sample may be used. If the sample is a mineral, fuse it with sodium sulfite and take up the mass directly with aqua regia, thereby causing precipitation of most of the  $\text{H}_2\text{WO}_4$  along with  $\text{SiO}_2$ . If the sample is an alloy, treat with aqua regia directly. The separation of tungsten is not quantitative, because of the presence of metatungstic acid. Treat the filtrate, which should contain iron (10 per cent is

<sup>1</sup>A. Travers, *Compt. rend.*, **166**, 416 (1918); *cf. ibid.*, **165**, 408 (1917).

sufficient), with ammonium hydroxide until it is just alkaline to litmus. The ferric hydroxide thus precipitated entrains the tungstic acid. Wash the precipitate free from sodium salts, dissolve it on the filter with 6 N hydrochloric acid, and add it to the major part of the  $H_2WO_4$ , after the latter has been freed from  $SiO_2$  by any of the usual methods and taken up in hydrochloric acid. Evaporate the combined solutions to a volume of about 2 cc., cool, dilute to 40 cc., add 5 cc. of the titanium chloride solution, dilute to 50 cc., mix, and compare at once with the standard by the method of dilution or balancing.

#### Notes.

1. In the case of both standard and sample, the final comparison solution should not contain over 1 mg. of tungsten per cubic centimeter, otherwise flocculation of the colloidal oxide becomes too rapid.

2. The reaction is not applicable in the presence of vanadium, phosphorus, or molybdenum, and hence these must be first removed.

3. Up to 0.1 N hydrochloric acid in the final comparison solution causes no appreciable change in the color. Beyond this concentration the color diminishes progressively, being completely eliminated in a solution 0.5 N in acidity.

4. The comparisons should be made at once after adding the  $TiCl_3$ , diluting and mixing, although the colloidal suspension will usually remain stable 30 minutes or longer under the conditions of the procedure.

### DETERMINATION OF VANADIUM IN IRON AND STEEL

#### METHOD OF McCABE<sup>2</sup>

The method is based upon the intense red-brown color obtained when hydrogen peroxide is added to an acid solution of vanadium in the pentavalent state. Since the color obtained in iron and steel analysis is a blend of the color due to ferric iron and that due to the vanadium, it is necessary to have the same weight of iron and the same volume of solution of both sample and standard. This condition is conveniently accomplished by imitating the color of the sample solution by adding a measured quantity of a standard vanadium solution to that of a non-vanadium steel, adjusting both solutions to the same volume before the final comparison.

<sup>2</sup>J. Ind. Eng. Chem., 5, 736 (1913).

**Reagents.**

1. Sulfuric acid, 9 N. Add 1 volume of sulfuric acid, sp. gr. 1.84, to 3 volumes of water.
2. Nitric acid, sp. gr. 1.42.
3. Potassium permanganate.
4. Potassium bichromate.
5. Ammonium bisulfite.
6. Hydrogen peroxide, 3 per cent. Determine the approximate strength of the peroxide by titration against a standard permanganate solution. This should be done every few weeks, otherwise serious error may arise due to deterioration of the peroxide.
7. Standard vanadium solution. The pure vanadium pentoxide required for making the standard solution can be obtained in the market, or it may be prepared in the laboratory from iron vanadate according to the method given by McCabe.<sup>3</sup>

Weigh out accurately about 0.2 gram of pure vanadium pentoxide, place it in a small beaker, add 30 cc. of nitric acid (sp. gr. 1.2) and warm until solution is complete. Dilute so that 1 cc. contains 0.2 mg. of vanadium, or an amount corresponding to 0.01 per cent when a 2-gram sample is taken for analysis.

The following procedures are from the work of McCabe.<sup>3</sup>

**Procedure A: When Chromium is Absent.**—Place 2 grams of the vanadium steel and the same quantity of a non-vanadium steel in 300 cc. Erlenmeyer flasks. Add exactly 40 cc. nitric acid (sp. gr. 1.2) to each and heat until the steel is dissolved. To each add about 0.1 gram potassium permanganate and digest for 4 minutes. Then add dilute ammonium bisulfite in sufficient quantity to clarify the solution, and continue heating until SO<sub>2</sub> is all expelled: by this operation carbon is oxidized.

Cool the solutions in running water and transfer to comparing tubes. Bring to equal volumes if they are not already so, and mix. Observe the colors, which should be of the same character and depth.

Into each tube introduce exactly 1 cc. 3 per cent hydrogen peroxide. Mix the test solution, and observe the vanadium color to gain an approximate idea of the vanadium content of the test.

To the solution of non-vanadium steel add, from a burette, as much of the standard vanadium solution as it is thought may be safely used

<sup>3</sup> *Loc. cit.*

without passing the amount existing in the test solution. To the test solution add an equal amount of water. Mix the solutions and compare colors. If the test solution appears darker, as will be the case if the proper precaution has been observed, add a further quantity of standard vanadium solution to the non-vanadium steel solution. Add an equal quantity of water to the test solution to maintain the equality of volume. Mix, and again compare. Continue in this manner until the colors match at equal volumes. The amount of standard vanadium solution used indicates the result. Thus, if 20.5 cc. standard vanadium solution are used, the result is 0.205 per cent vanadium.

**Procedure B: When Chromium is Present.**—Weigh 2 grams of the chrome-vanadium steel and an equal amount of a plain steel, placing in 400 cc. beakers. Weigh accurately an amount of potassium bichromate corresponding to the chromium content of the test steel and place it in the beaker containing the plain steel. To each add 80 cc. sulfuric acid (1:3), and heat.

When nearly in solution, add to each 25 cc. of strong nitric acid and heat for 10 minutes. Vanadium is not completely oxidized by a small amount of nitric acid such as would oxidize the iron.

Cool the two solutions in running water, introduce into comparing tubes, and bring to equal volumes. Mix, and observe the colors. Owing to possible error in the chromium determination, it is doubly important to know that the solutions show colors of the same character and depth at equal volumes before proceeding with the analysis.

The determination is now finished as when chromium is absent.

#### Notes.

1. The above procedures are entirely satisfactory for the determination of vanadium in most steels and iron, but when titanium or molybdenum are present, or when there is a small amount of vanadium present with a large amount of chromium, the more elaborate method of McCabe should be used. This method<sup>4</sup> requires more time, but has been found thoroughly reliable under adverse conditions.

2. The rules governing all color methods are of unusual importance in the colorimetric determination of vanadium. The large sample necessary renders the iron color deep enough to influence the vanadium color very materially. The determination is further lim-

<sup>4</sup> Chem. Eng., 13, 243 (1911); see also the following method, page 391.

ited by the sensitive character of the vanadium color with hydrogen peroxide, it being affected by the acidity of the solution. With the strength of acids commonly used in analytical work, the acidity may be satisfactorily controlled by means of the ordinary volumetric apparatus. An excess of hydrogen peroxide partially bleaches the color after having produced it. Also, it is only when vanadium is in the pentavalent state that hydrogen peroxide imparts to its solution an immediate lasting color.

3. It is very important to boil thoroughly the solution after using the ammonium bisulfite. This is necessary in order to restore the solution to the fully oxidized condition. Any reduced vanadium imparts no color to the solution.<sup>5</sup>

**MCCABE'S METHOD FOR THE DETERMINATION OF VANADIUM IN IRON AND STEEL CONTAINING TUNGSTEN, CHROMIUM, TITANIUM, AND MOLYBDENUM<sup>6</sup>**

The principle is the same as that of the preceding method.

**Reagents.**

1. Nitric acid, sp. gr. 1.42.
2. Hydrochloric acid, sp. gr. 1.19.
3. Hydrofluoric acid, 48 per cent. Use the pure acid in ceresin bottles.
4. Ammonium hydroxide, sp. gr. 0.90.
5. Hydrogen peroxide, 3 per cent.
6. Ether. Use alcohol-free ether.
7. Potassium chlorate.
8. Standard vanadium solution. Prepare a solution containing 0.2 mg. of vanadium per cubic centimeter. Accurately weigh about half a gram of pure  $V_2O_5$ , dissolve it in a minimum amount of nitric acid, dilute to the desired concentration, and mix thoroughly.

**Procedure.**—Accurately weigh out about a 2-gram sample. If tungsten is present, remove it in the usual manner but avoid using sulfuric acid. Evaporation to dryness in the tungsten determination reduces a portion of the vanadium. It is therefore necessary to reoxidize it during the evaporation of the filtrate preceding the ether sep-

<sup>5</sup> Private communication from Mr. C. R. McCabe.

<sup>6</sup> Private communication from Mr. C. R. McCabe; see also McCabe, Chem. Eng., **13**, 243 (1911).

aration of the bulk of the iron. To the filtrate add 5 cc. of conc. nitric acid and evaporate to about 10 cc. Pour into a separatory funnel, and wash the dish or beaker with hydrochloric acid (1:1), using a small quantity at a time until the volume of the solution amounts to 20 cc. as shown by a mark on the funnel. Add 50 cc. of alcohol-free ether, stopper tightly, and shake under the water-tap. Allow the funnel to stand in a rack until the two layers separate completely. Draw off the lower layer (acid layer) and take about 1.5 cc. of the ethereal solution containing the iron along with the acid solution. This yields about 0.1 gram of iron, together with the vanadium, chromium, and titanium if present. Molybdenum, if present, remains in the ethereal solution.

Evaporate the acid solution nearly to dryness, add conc. nitric acid and evaporate again nearly to dryness, thus removing all chlorine. Add 20 cc. of conc. nitric acid, boil, add 2 grams of potassium chlorate and continue boiling a moment or two. The chromium and manganese are oxidized. Dilute to about 250 cc. and while boiling cautiously add ammonia in excess. (See Note 3.) *The precipitated iron occludes the vanadium*, chromium passing into the solution. Any titanium present is with the ferric hydroxide precipitate. Filter on a 9 cm. filter paper and wash with hot water. Open the paper in a dish, add 3 cc. of 1:1 hydrochloric acid and 5 cc. of water and warm until solution is complete. *Avoid any more acid at this point.* Filter the solution to remove any paper fiber and also the small amount of manganese which may separate at this point. This filtration is not of vital consequence. The volume of the solution is now 40 to 50 cc. Transfer the solution to a color comparison tube. The solution has the color of ferric chloride solution. Add 0.5 to 1 cc. of hydrofluoric acid and mix. The ferric chloride color is discharged, leaving the solution clear and colorless. (This would not be the case if much hydrochloric acid were present.)

Add 1 cc. of 3 per cent hydrogen peroxide. The characteristic brown color is developed in the presence of the merest trace of vanadium. Comparison of the color is made with the standard  $V_2O_5$  solution as described on page 389.

#### Notes.

1. Titanium comes through with the vanadium, but the hydrofluoric acid prevents any color developing. The true vanadium color

is developed only in the absence of any interfering or obscuring colors. McCabe's original paper directed the use of a non-vanadium steel to be carried along with the vanadium steel, the final solution being used with  $V_2O_5$  as a standard. This is now considered unnecessary, as there are no interferences to be compensated.

2. In making the color comparison, use only a dilute hydrochloric acid solution of the same concentration as that containing the vanadium, and add the  $V_2O_5$  solution until the colors match at *equal volumes*.

3. The manganese tends to dissolve upon diluting and heating the solution after the potassium chlorate treatment.

#### DETERMINATION OF VANADIUM BY STRYCHNINE

When a solution of strychnine in concentrated sulfuric acid is added to a concentrated sulfuric acid solution of vanadium in the highest state of oxidation ( $V_2O_5$ ), a deep violet color is produced, which gradually changes to an intense orange. The reaction is carried out in the cold ( $20^\circ \text{C.}$ ) and 10 minutes is required for the maximum intensity of the orange tint to develop.

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Nitric acid, 6 N.
3. Ammonium hydroxide, sp. gr. 0.90.
4. Disodium hydrogen phosphate, 1 N.
5. Potassium chlorate.
6. Ammonium molybdate. 100 grams of molybdic acid ( $\text{MoO}_3$ ) are stirred into 400 cc. of cold water, 80 cc. of ammonium hydroxide (sp. gr. 0.90) added, and the solution filtered. Pour the filtrate slowly, with constant stirring, into a nitric acid solution prepared by diluting 400 cc. of nitric acid (sp. gr. 1.42) with 600 cc. of water. Add 0.05 gram of microcosmic salt, allow the solution to stand 24 hours, and filter.
7. Strychnine reagent. Dissolve 4 grams of pure strychnine in a liter of sulfuric acid (sp. gr. 1.84).
8. Standard vanadium solution. Dissolve 0.1785 gram of pure vanadium pentoxide in a small volume of concentrated sulfuric acid and then dilute to a liter with sulfuric acid (sp. gr. 1.84). Mix thoroughly. One cubic centimeter of this solution contains 0.1 mg. of vanadium.



**Procedure.**—Dissolve the sample in 40 cc. of 6 N nitric acid and add 1 cc. of the sodium acid phosphate solution. Cool, and add, a little at a time, concentrated ammonium hydroxide until ferric hydroxide just begins to appear and then boil until it redissolves. Remove from the flame, add 30 cc. of ammonium molybdate solution, stir thoroughly, filter, and wash the precipitate with 2 per cent nitric solution until free from iron, and then wash it with water. Transfer the precipitate to a large beaker, add a few crystals of potassium chlorate, 20 cc. of sulfuric acid (sp. gr. 1.84), and evaporate until dense white fumes of sulfuric acid are given off and chlorine dioxide is no longer evolved.<sup>7</sup> Cool to room temperature, transfer to a Nessler cylinder, the beaker being rinsed out with a little concentrated sulfuric acid, add 20 cc. of strychnine reagent, allow the solution to stand 10 minutes and compare with a standard by the method of dilution.

The standard comparison solution is prepared along with the sample by taking a volume of the standard vanadium solution containing the amount of vanadium estimated to be in the sample and treating it with potassium chlorate and sulfuric acid as directed. The cooled solution is transferred to a Nessler cylinder of the same bore as the one containing the sample and 20 cc. of strychnine reagent added. After standing 10 minutes, the solutions are compared and the one having the more intense color is then diluted with concentrated sulfuric acid until the tints in the two tubes match when viewed against a semi-opaque white glass screen.

#### Notes.

1. For the best results the amounts of vanadium in sample and standard should be approximately the same.

2. Nessler cylinders 12 ins. tall and  $\frac{3}{4}$  in. internal diameter are convenient for comparison. With these, 0.5 mg. of vanadium gives a good comparison tint when diluted until the solution fills about one-half the volume of the cylinder.

3. The vanadium must be separated from iron, since the latter interferes with the color comparison and, when present in a large amount, entirely prevents the formation of the color.

4. "The presence of large quantities of titanium, molybdenum, tungsten, or aluminum do not affect the test."<sup>8</sup>

<sup>7</sup> Cf. A. W. Gregory, *Chem. News*, **100**, 221 (1909).

<sup>8</sup> A. W. Gregory, *loc. cit.*

5. The vanadium must be in the highest state of oxidation ( $V_2O_5$ ).
6. Gregory<sup>9</sup> tested the permanency of the color, using 1 mg. of vanadium, and could not detect any change after letting it stand 3 hours and then comparing it with the same quantity of vanadium freshly treated.
7. Comparison by duplication is not practicable since 10 minutes are required for the full intensity of the orange color to develop after the strychnine reagent is added. The large volume of solution required for the balancing method makes the latter unsatisfactory on account of expense. The color will in time fade, and hence a series of standards is not practicable.

<sup>9</sup> *Loc. cit.*

## CHAPTER XXXV

### ZINC

#### DETERMINATION OF ZINC BY RESORCINOL

THE method is based upon the blue color imparted to an ammoniacal zinc solution when a small quantity of an alcoholic or ethereal solution of resorcinol is added.<sup>1</sup>

#### Reagents.

1. Nitric acid, 6 N.
2. Ammonium hydroxide, 6 N.
3. Resorcinol, 5 per cent solution in alcohol or ether.
4. Standard zinc solution. Dissolve 0.1 gram of pure zinc in nitric acid and dilute to 1 liter.

**Procedure.**—The sample taken for analysis should contain between 0.005 and 3 mg. of zinc. It is dissolved (in acid if necessary) and ammonium hydroxide added until the precipitate formed redissolves. The solution is diluted to 100 cc., and 2 cc. of the resorcinol solution added. The blue color thus produced is matched against a standard prepared in the same way and containing about the same quantity of zinc as the sample. The dilution method holds only over a very narrow range. The method of duplication may also be used but not the other methods.

#### Notes.

1. The reaction employed in the above analysis is sensitive to 1 part in 100,000. Errors observed for concentrations of zinc between 0.1 and 3.2 mg. per 100 cc. varied from 0.06 to 6.6 per cent.
2. The character of the blue color of the solution is slightly altered

<sup>1</sup> A. del Campo y Cerdan and J. de la Puente, *Anales soc. españ. fis. quim.*, **98**; Cerdan, *Ann. chem. anal. appl.*, **14**, 205.

on exposure to air. Unless the comparison is made at once, the solutions must be protected by a layer of some suitable oil. "Nujol" oil is excellent for this purpose.

3. Hydrochloric acid solutions of zinc are colored red by resorcinol. They are not altered by air, but a special concentration curve must be constructed, using known amounts of zinc in the same way as the sample to be treated. See Chapter IV.

4. In ammoniacal solution, calcium gives a green coloration with resorcinol, nickel a blue, and cobalt a red. Hence, it is necessary to remove these metals before testing for zinc.

5. Zinc borosilicate glassware should not be used. See page 719.

#### DETERMINATION OF ZINC BY THE POTASSIUM FERROCYANIDE METHOD (TURBIDIMETRIC METHOD)

This method is based upon the turbidimetric method described by Breyer<sup>2</sup> in which zinc is precipitated as a finely divided suspension by potassium ferrocyanide solution. Meldrum<sup>3</sup> has applied it to the determination of zinc in water, and Birckner<sup>4</sup> to the determination of zinc in various food products. Bodansky<sup>5</sup> has modified the Breyer-Birckner method and used it in the estimation of the zinc content of marine organisms. Solid calcium citrate is employed in the recovery of the colloidal zinc sulfide. His modified method is given here.

#### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Nitric acid, sp. gr. 1.42.
3. Hydrochloric acid, sp. gr. 1.19.
4. Hydrochloric acid, 6 N.
5. Citric acid, 50 per cent solution.
6. Ammonium hydroxide, 6 N.
7. Hydrogen sulfide, thoroughly washed. See p. 240,
8. Calcium carbonate.
9. Ammonium thiocyanate, 2 per cent solution.
10. Potassium ferrocyanide, 34.8 grams per liter.

<sup>2</sup> See W. W. Scott, *Standard Methods of Chemical Analysis*, 4th ed., p. 607. D. Van Nostrand Co., New York, 1925.

<sup>3</sup> *Chem. News*, **116**, 271, 295, 308 (1917).

<sup>4</sup> *J. Biol. Chem.*, **88**, 191 (1918).

<sup>5</sup> *J. Ind. Eng. Chem.*, **13**, 696 (1921).

11. *Standard zinc solution.* Dissolve 1 gram of pure zinc (iron- and lead-free) in dilute HCl, evaporate to a small volume, dilute, filter, make up to a liter, and thoroughly mix. From this a weaker standard is made, containing 0.1 mg. per cubic centimeter. The standard may also be made with recently ignited pure  $\text{ZnCO}_3$ , or  $\text{ZnC}_2\text{O}_4$ .

The standard solutions must be slightly acid.

**Procedure.**—A weighed amount of the material containing between 0.1 and 5 mg. is treated with concentrated  $\text{H}_2\text{SO}_4$  and concentrated  $\text{HNO}_3$  to oxidize the organic matter, the acids evaporated off and the residue washed. The ash is repeatedly extracted with hot dilute HCl, the filtered extracts being collected in a casserole and evaporated to dryness. Dissolve the residue in 2 cc. of concentrated HCl and 50 cc. of water. Pass  $\text{H}_2\text{S}$  through the solution until the copper is completely precipitated as sulfide, filter, and wash the precipitate. Boil the filtrate until all the  $\text{H}_2\text{S}$  has been expelled (test with lead acetate paper), cool, neutralize with ammonium hydroxide, and add 10 cc. of 50 per cent citric acid solution. Heat to boiling. If no calcium citrate separates, add calcium carbonate, a little at a time, until about 1 gram of calcium citrate is formed. Hydrogen sulfide is then passed rapidly into the solution until the latter has cooled. Allow the solution to stand for several hours, part of the time on a water-bath, until the supernatant liquid is clear. Collect the precipitate on a filter, wash with 2 per cent ammonium thiocyanate, and dissolve the residue on the filter paper with hot dilute HCl, allowing the filtrate to run into the flask in which the precipitation was made. If the solution is reddish in color due to ferric thiocyanate, the zinc is reprecipitated. A turbidity due to colloidal sulfur may be removed by boiling and filtering off the coagulated sulfur. If clear and colorless, the liquid is ready for making the turbidimetric comparison. The liquid, or an aliquot part, is put into a 50 cc. Nessler cylinder and diluted to 45 cc. with water. At the same time a series of Nessler cylinders is prepared, containing varying quantities of zinc, 3 cc. of concentrated HCl and diluted to 45 cc. with water. Five cubic centimeters of potassium ferrocyanide solution are then added to each cylinder. The solutions are quickly mixed and the turbidities compared at once by viewing the cylinders longitudinally when they are held over a sheet of dead black paper or a board painted dead black. Standard zinc solution may be added from a burette to the cylinder which almost matches the unknown, until finally the two turbidities are identical.

### Notes

1. According to Birkner (*loc. cit.*), who used the potassium ferrocyanide method for the determination of zinc in various food products, the results are accurate to  $\pm 0.05$  mg., if the procedure is properly carried out. Meldrum (*loc. cit.*) found the method accurate in water analysis to 1 part of zinc in a million parts of water.

2. Iron must be removed before the addition of potassium ferrocyanide, since ferrous salts would form potassium ferri-ferrocyanide which imparts a blue color to the solution.

3. A better recovery of the zinc sulfide is obtained when the calcium citrate is formed in the solution than when it is added pre-formed. It is thought that the calcium citrate adsorbs the colloidal zinc sulfide particles and hence effects a better recovery. This is in line with Bancroft's observation:<sup>6</sup> "There is some evidence to show that when a colloidal solution is precipitated the finer particles attach themselves to the coarser ones."

4. The following data taken from the results of Bodansky (*loc. cit.*), illustrate the degree of accuracy of the method when solid calcium citrate is used in the recovery of the finely divided zinc sulfide:

TABLE XXXVII

Zn Present, Milligram	Zn Found, Milligram	Zn Present, Milligrams	Zn Found, Milligrams
0.10	0.11	0.50	0.48
0.20	0.18	1.00	0.99
0.30	0.28	2.50	2.40
0.40	0.39	10.00	10.40

This method is excellent for the estimation of less than 5 mg. of zinc; and even with larger amounts, 10 to 20 mg., it compares favorably with other standard methods.

5. The zinc ferrocyanide should give to the solution a pure milky white opalescence. Unless the greatest precaution is taken to remove all iron, the zinc ferrocyanide appears a very faint bluish white due to the minute trace of iron which forms ferri-ferrocyanide. (See Note 2.)

6. Zinc borosilicate glassware should not be used. See page 719.

<sup>6</sup> J. Ind. Eng. Chem., **13**, 153 (1921); cf. Burton, The Physical Properties of Colloidal Solutions, 2d ed., p. 173. Longmans, Green and Co., London, 1921.



## PART III

### ORGANIC

#### CHAPTER XXXVI

#### ACETALDEHYDE, ACETYLENE, AND ACROLEIN

##### DETERMINATION OF ACETALDEHYDE BY FUCHSINE-SULFUROUS ACID

###### Reagents.

1. Aldehyde-free alcohol. Commercial 95 per cent alcohol is first treated with silver oxide as described by Dunlap<sup>1</sup> and the distillate then treated with meta-phenylenediamine hydrochloride according to Woodman and Lyford.<sup>2</sup> The combined method is given on page 412 under the Determination of Benzaldehyde.

2. Fuchsine-sulfurous acid. Dissolve 0.5 gram of pure fuchsine in 500 cc. of water, add 5 grams of sulfur dioxide dissolved in 100 to 200 cc. water, dilute to a liter, and allow to stand until the solution is colorless. Prepare only a small quantity of this reagent at a time since it loses its strength in a few days.

3. Standard acetaldehyde solution. Prepare according to Vasey.<sup>3</sup> Aldehyde ammonia is ground in a mortar with ether, the ether decanted and the operation repeated several times. The purified substance is dried in a current of air and then in a vacuum over concentrated sulfuric acid. Dissolve 1.387 grams of the purified aldehyde ammonia in 50 cc. of 95 per cent (by volume) alcohol, add 22.7 cc. of normal alcoholic sulfuric acid, dilute to 100 cc. and then add 0.8 cc. more water to compensate for the volume occupied by the ammonium sulfate precipitate. Let the mixture stand overnight and filter through a dry filter. This solution contains 1 gram of acetaldehyde per 100 cc. and will not lose its strength.

<sup>1</sup> J. Am. Chem. Soc., **28**, 395 (1906).

<sup>2</sup> *Ibid.*, **30**, 1607 (1908).

<sup>3</sup> Analysis of Potable Spirits, p. 30.



A convenient standard is prepared by diluting 2 cc. of the strong solution to 100 cc. with 50 per cent (by volume) alcohol. One cubic centimeter of the dilute solution contains 0.2 mg. of acetaldehyde. This solution loses strength fairly rapidly and should be made up fresh every day or two.

**Procedure.**—Five or 10 cc. of the sample solution, which should contain 0.5 to 1.5 mg. of acetaldehyde, are placed in a colorimeter tube, diluted to 50 cc. with 50 per cent (by volume) alcohol (aldehyde-free), the tube placed in a water-bath at 15° and allowed to reach the temperature of the bath. Twenty-five cubic centimeters of the fuchsine-sulfurous acid solution (also at 15°) are added and the two solutions thoroughly mixed and allowed to stand in the bath for 15 minutes. The solution is then quickly matched in a colorimeter against a standard acetaldehyde solution similarly treated, care being taken that both sample and standard are at the same temperature. The room temperature should not be much above 15°.

The intensity of color developed by acetaldehyde under the above conditions is not in direct proportion to the amount of aldehyde present. "A standard containing 0.001 gram develops about three times as much color as a standard 0.0005 gram and about one-half the color of a standard containing 0.0015 gram."<sup>4</sup> This makes it necessary to prepare a number of curves and from these to prepare a table.

Using a Schreiner<sup>5</sup> colorimeter, Tolman<sup>6</sup> has prepared such a table and gives directions for using it.

#### DETERMINATION OF ACETYLENE BY AMMONIACAL CUPROUS CHLORIDE

##### METHOD OF WEAVER<sup>7</sup>

This method is based upon the formation of a red colloidal solution of copper carbide when acetylene is conducted into an ammoniacal solution of cuprous chloride containing gelatin and alcohol. The gelatin is added as a protective colloid to retard precipitation of the colloidal copper carbide with a resultant change in color. The sample sol is matched in color against a standardized red dye solution or a cal-

<sup>4</sup> L. M. Tolman, *J. Am. Chem. Soc.*, **28**, 1624 (1906).

<sup>5</sup> *J. Am. Chem. Soc.*, **27**, 1192 (1905).

<sup>6</sup> *Loc. cit.*

<sup>7</sup> U. S. Bureau of Standards, *Sci. Papers No. 267* (1916).

ibrated piece of ruby glass. Colloidal solutions of copper carbide are not sufficiently stable to be conveniently used as standards.

#### Reagents.

1. Ammonium hydroxide, sp. gr. 0.90.
2. Cuprous chloride.
3. Hydroxylamine hydrochloride.
4. Gelatin.
5. Alcohol, 95 per cent.
6. Standard color solution. Dissolve 0.21 mg. of chromanilbraun R, 0.04 mg. of carmoisine B, and 2.5 grams of gum arabic in 100 cc. of water.

**Procedure.**—Dissolve 0.25 gram of gelatin in hot water, dilute to 500 cc., and add 500 cc. of 95 per cent alcohol and 1.25 grams of hydroxylamine hydrochloride. To 20 cc. of this solution add 10 cc. of concentrated ammonium hydroxide and a small amount (0.01 to 0.02 gram) of cuprous chloride. After the absorption of the acetylene the solution is diluted to 100 cc. and compared in a colorimeter with the standard which has been chosen. The standard used by Weaver in his experimental work was a solution containing chromanilbraun R, carmoisine B, and gum arabic. A more convenient, though less accurate, standard is a fixed depth of a solution of azolitmin. If 10 cm. of a solution of azolitmin containing 1 part of the dye to 2500 parts of water is used as standard, the amount of acetylene in 100 cc. of colloidal solution may be calculated from the equation  $x = 0.13y + 0.03$ , where  $x$  = number of milligrams of acetylene and  $y = 10 \div$  number of centimeters of colloidal solution required to match the standard.

Weaver<sup>8</sup> used a colorimeter similar to the one described by Campbell and Hurley.

#### Notes.

1. The method is very sensitive. Amounts of acetylene as small as 0.03 mg. may be detected, and amounts up to 2 mg. may be determined with an accuracy of better than 0.05 mg.
2. Hydrogen sulfide and large amounts of oxygen and carbon dioxide interfere with the test, but all of these may be removed, without loss of acetylene, by passing the gas to be tested through a hot alkaline solution of pyrogallol.

<sup>8</sup> J. Am. Chem. Soc., **33**, 1112 (1911).

3. The following statements are by Weaver,<sup>9</sup> who made a careful investigation of the effects of all variables in the above colorimetric method:

(a) *Size of Tip and Rate of Gas Flow.*—When the gas is introduced into the absorption solution through a tube with a fine tip about 0.2 mm. in diameter, curved so that bubbles escaping from the tip pass through the solution without coming in contact with the glass after leaving the tip, the indications are that the absorption of acetylene from the very fine bubbles is complete. The results are independent of the rate of gas flow at rates of 5 to 50 cc. per minute. Even at a rate of 100 cc. per minute, the loss amounts to only about 10 per cent.

(b) *Composition of Absorbing Solution.*—Tests were made to determine the influence of the following constituents in the solution used for absorption: cupric chloride, ammonium chloride, hydroxylamine hydrochloride, cuprous chloride, ammonia, gelatin, alcohol, and acetone.

In the following discussion of results the amount of each of the various constituents mentioned as present in the solution is always the amount contained in 30 cc.

**Cupric Chloride.**—It was found that the presence of even quite large amounts of cupric chloride in the absorbing solution is without appreciable effect, provided a sufficient amount of the cuprous salt is also present during the absorption and enough hydroxylamine is added before the colorimetric comparison to reduce all cupric salts. Cupric chloride may be added to a colloidal solution already prepared and left for several hours without affecting the colloid, as shown by colorimetric tests after reducing the cupric salts with hydroxylamine. If the solution is exposed to the air the colloid is unaffected until the cuprous salts in solution are oxidized; then the colloid quickly disappears with the formation of a flocculent black precipitate.

**Ammonium Chloride.**—The presence of ammonium chloride, or, indeed, any strong electrolyte, causes irregular and generally low results. Ten milligrams of the salt in 30 cc. of absorbing solution produce a tendency toward low results. Twenty milligrams cause results averaging 40 per cent low, and more than that amount cause precipitation. The presence of ammonium chloride in the absorbing solution is the most serious single source of error in the colorimetric

<sup>9</sup> *Loc. cit.*

determination of acetylene. After its effect was discovered, solutions showing the characteristic brownish and slightly turbid appearance caused by the presence of much of the salt were discarded.

**Hydroxylamine Hydrochloride.**—The presence of a small amount of hydroxylamine hydrochloride in excess of that required to decolorize the absorbing solution is without appreciable effect, but a large excess has the same effect as a small amount of ammonium chloride, e.g., 100 mg. of hydroxylamine hydrochloride caused the average results to be about 10 per cent low.

**Cuprous Chloride.**—Only a very small amount of cuprous chloride is required to give complete absorption of the acetylene, and the amount present may be varied over a wide range without affecting the results. Solutions containing from 9 to 360 mg. gave identical results. Solutions containing 6 mg. gave results about 30 per cent low, although the amount of acetylene was much less than equivalent to the amount of copper, while solutions containing only 3 mg. of cuprous chloride gave no color at all.

**Ammonia.**—The amount of ammonia in solution must be regulated rather carefully. About 10 cc. of concentrated ammonium hydroxide (sp. gr. 0.90) per 30 cc. of solution gives the best results. Irregular results are caused by any considerable change in the concentration in either direction. The use of only 5 cc. caused results 40 per cent low, while the use of 2.5 cc. gave results 60 per cent low. More than 10 cc. of strong ammonium hydroxide is likely to produce a cloudy appearance caused by coagulation of the gelatin; 20 cc. always causes the formation of a large amount of precipitate, sometimes enough to leave the solution practically colorless.

**Gelatin.**—The amount of gelatin may be varied from 2 to 6 mg. per 30 cc. provided coagulation does not take place. The amount should be kept small to prevent coagulation. Less than 1 and more than 10 mg. is almost certain to cause precipitation.

**Alcohol.**—The presence of a large amount of alcohol favors uniform results, but alcohol causes gelatin to coagulate and the amount which can be used is limited by this fact. About 10 cc. of 95 per cent alcohol per 30 cc. of solution gave the most favorable results. When only 5 cc. is used the results are about 30 per cent low. Less than 5 cc. causes precipitation. Too great a variation of the alcohol concentration in either direction causes precipitation.

**Acetone.**—The effect of substituting acetone for a part or all of the

alcohol was tried. In general, the color of the resulting colloid was changed and comparisons with the standard were difficult. Acetone also coagulates gelatin more readily than alcohol.

(c) *Temperature*.—A few experiments on the effect of temperature were carried out. The results show that the temperature changes in the ordinary laboratory do not affect the result appreciably. There is a tendency for the results to be low at temperatures above 35° C. Results at 45° to 50° are 10 to 15 per cent low.

(d) *Dilution of Solution after Absorption*.—A large number of experiments have shown that, within the limit of accuracy of the colorimeter readings, it is immaterial how much the colloidal solution is diluted before the readings are taken, provided the volume of solution is taken into account in calculating the amount of acetylene. For example, if a sample of acetylene is absorbed in 30 cc. of solution and the resulting liquid successively diluted with water to 60, 90, and 120 cc. and comparisons with the same color standard are made at each dilution, it will be found that the depths of colloidal solution required to match the standard are in the ratios 1 : 2 : 3 : 4.

4. A solution of acetylene in ether is a convenient and suitable source of acetylene. A uniform flow of gas may thus be easily obtained.

5. The constituents normally present in illuminating gas do not interfere with the colorimetric determination of acetylene.

6. The method can be used to determine acetylene in air. The acetylene-air mixture is passed through a strongly alkaline solution of pyrogallol and thence into the absorption solution. The pyrogallol solution is finally heated to boiling to drive out any acetylene present.

7. Potassium or sodium hydroxide may be used if necessary to remove hydrogen sulfide, carbon dioxide, or similar interfering gases. Small amounts of carbon dioxide do not interfere but large amounts have the same effect as the introduction of a little ammonium chloride.

#### REFERENCES

1. Berthelot, *Compt. rend.*, **54**, 1070 (1862). Seems to have been the first to use an ammoniacal solution of a cuprous salt to detect acetylene.
2. Ilosvay, *Ber.*, **32**, 2697 (1899). Prepared cuprous salts by reduction of cupric salts with hydroxylamine.
3. Küspert, *Z. anorg. Chem.*, **34**, 453 (1903). Observed that cuprous chloride gives a red colloid when added to an aqueous solution of acetylene.
4. Schulze, *Z. angew. Chem.*, **29**, I, 341 (1916). Uses ammoniacal cuprous chloride solution. Apparently not aware of Weaver's (*loc. cit.*) work.

**DETERMINATION OF ACROLEIN BY FUCHSINE-SULFUROUS ACID**

The acrolein is determined by the same procedure used for the estimation of benzaldehyde by Schiff's reagent. The same precautions are taken as are required in the benzaldehyde test. (See p. 412.)

Moureu and Boismenu<sup>10</sup> recommended constructing a graph from known quantities of acrolein as abscissas and intensities of color as ordinates. The concentration of acrolein in the sample may then be read off the graph after the color intensity has been determined. In diluting a solution of acrolein, the coloring power of Schiff's reagent diminishes faster than the concentration.

The use of stabilizers for acrolein, such as pyrogallol, pyrocatechol, hydroquinol, gallic acid, and tannin, does not interfere with the determination.

<sup>10</sup> J. pharm. chim., 27, 49, 89 (1923).

## CHAPTER XXXVII

### ADRENALINE

#### DETERMINATION OF ADRENALINE

WHEN solutions of adrenaline (epinephrine or suprarénine),  $C_6H_3(OH)_2 \cdot CH(OH) \cdot CH_2 \cdot NH \cdot CH_3$ , are oxidized by heating with potassium iodate a reddish color develops which varies in intensity with the concentration of the adrenaline. The method is especially adapted to the estimation of adrenaline in glands. If the procedure is carried out on solutions of known acidity and with an allowance, if necessary, for the presence of bisulfite, the results will be found sufficiently reliable.

#### Reagents.

1. Hydrochloric acid, 1 N.
2. Potassium iodate, 1 per cent.
3. Sodium bisulfite.
4. Standard adrenaline solution. Prepare freshly as needed. Place 0.050 gram of pure adrenaline in a 50 cc. volumetric flask, dissolve it in 0.5 cc. of N hydrochloric acid, dilute to the mark with water, and thoroughly mix. If the sample to be compared contains bisulfite, then 0.050 gram of sodium bisulfite should be added to the above standard solution before diluting to the mark.

#### ADRENALINE IN SOLUTIONS

**Procedure.**—Place 20 cc. of distilled water in a small flask (50 cc.), add 5 cc. of a 1 per cent solution of potassium iodate and 0.25 cc. of N hydrochloric acid, and warm to  $38^\circ C$ . Then add 0.5 cc. of standard adrenaline solution, and continue heating for 15 minutes. At the same time prepare an acid solution of the iodate in the same way, warm to  $38^\circ C$ ., and add 0.5 cc. of the sample solution (approx. concn. 1 : 1000), or an equivalent amount of a weaker solution. After the standard and sample solutions have been warmed for 15 minutes at about  $38^\circ$

C., they are cooled to room temperature and compared in a colorimeter or Nessler cylinders.

#### ADRENALINE IN DRIED GLANDS

**Procedure.**—Digest 0.100 gram of the dried glands for about 30 minutes at 38° C. in 20 cc. of distilled water to which have been added 5 cc. of a 1 per cent potassium iodate solution and 0.25 cc. of N hydrochloric acid. Filter, cool, and match the solution against a standard solution prepared in a similar way at the same time. The standard adrenaline solution should not contain any bisulfite.

#### Notes.

1. If the sample solution is within 20 or 25 per cent of the standard, comparison is easily made; otherwise, it is better to repeat the analysis, using solutions of sample and standard approximately of the same concentration.

2. It has been shown that "the greatest intensity of color is reached in about 10 minutes at 38° C., and further heating at this temperature for an hour makes no appreciable difference. The presence of sodium bisulfite in the solution diminishes the color in proportion to the amount of bisulfite present. When the adrenaline solution (1 : 1000) contained a like amount of sodium bisulfite (0.1 per cent) the color was reduced 10 per cent."<sup>1</sup>

3. In attempting to match the color (produced in acid solution) with a mineral acid, Scoville found that an ammoniacal solution of cobaltous chloride matches closely within certain limits.

"A solution made by dissolving 0.25 gram of cobaltous chloride ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ) in 10 cc. of water, adding a solution of 2 grams of ammonium carbonate (U. S. P. in translucent crystals) in 80 cc. of water, and making the solution up to 100 cc., matches the adrenaline color very closely in depths of 25 to 35 mm. (in a Duboscq colorimeter) but shades more to the red in deeper columns and to the blue in shallower. The ammoniacal cobalt chloride solution therefore differs enough in color to limit its usefulness, but can be used to verify the claim for a 1 : 1000 solution of adrenaline, provided the test be applied with proper precautions."<sup>2</sup>

<sup>1</sup> W. L. Scoville, J. Ind. Eng. Chem., **12**, 770 (1920).

<sup>2</sup> W. L. Scoville, J. Ind. Eng. Chem., **12**, 771 (1920).



## CHAPTER XXXVIII

### ANILINE

#### DETERMINATION OF ANILINE VAPOR

WHEN a dilute solution of aniline is mixed with calcium hypochlorite solution and then made alkaline with caustic, a yellow color develops, whose intensity is proportional to the aniline present.

##### Reagents.

1. Sodium hydroxide, 1 N.
2. Calcium hypochlorite. Prepare a stock solution containing about 3 per cent of available chlorine by shaking for 10 minutes 20 grams of good commercial chloride of lime with 100 cc. of water and then filtering. A portion of this solution is diluted, as needed, to an available chlorine content of 0.1 per cent.
3. Standard aniline solution. Dissolve 1 gram of pure aniline in water, dilute to a liter, and mix thoroughly. One cubic centimeter of this solution contains 1 mg. of aniline.

**Procedure.**—The aniline vapor is collected by bubbling a measured volume of the air-aniline mixture through an absorption bulb containing water. A preliminary test is made to determine the approximate amount of aniline present and the solution then diluted with water to a concentration of 0.01 mg. to 0.1 mg. of aniline per 20 cc. One cubic centimeter of calcium hypochlorite (containing 0.1 per cent available chlorine) is then mixed with 20 cc. of the diluted solution. After standing 2 minutes, add 1 cc. of 1 N sodium hydroxide solution, allow to stand 10 minutes, and match the color against a series of standards similarly prepared from known amounts of the standard aniline solution. A suitable series may be made with 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10 mg. of aniline, respectively, in a volume of 20 cc. each, exclusive of reagents. The mixing of the unknown solution and the standards with the reagents should be made as nearly

simultaneously as possible. Fifty cubic centimeter-Nessler cylinders are convenient.

#### Notes.

1. It is important to mix the solutions in the order and manner directed in the Procedure.

2. The addition of alkali sensitizes the reaction between aniline and hypochlorite so that as little as 0.01 mg. of aniline in 20 cc. of solution (1 part in 2,000,000) can easily be detected.<sup>1</sup>

3. A much stronger (ten times) solution of aniline than 0.01 to 0.1 mg. per 20 cc. may be used, provided a set of standards with correspondingly higher aniline concentrations is employed. In the latter case the tubes should be allowed to stand a little longer than 10 minutes before comparing the colors.

<sup>1</sup> E. Elvove, J. Ind. Eng. Chem., **9**, 953 (1917).

## CHAPTER XXXIX

### BENZALDEHYDE AND CITRAL

#### DETERMINATION OF BENZALDEHYDE BY FUCHSINE-SULFUROUS ACID

THIS method is based upon the reaction of benzaldehyde with fuchsine-sulfurous acid, a pink color being formed, the intensity of which is proportional to the amount of benzaldehyde present. The method was developed for the estimation of benzaldehyde in almond extracts. With a properly prepared fuchsine-sulfurous acid solution, a distinct change in color is obtained when 0.05 mg. of benzaldehyde is present in a volume of 50 cc.<sup>1</sup>

#### Reagents.

1. Fuchsine-sulfurous acid. Dissolve 0.5 gram of pure fuchsine in 100 cc. of water and add a freshly prepared solution of sulfurous acid containing 20 grams of sulfur dioxide. As soon as the solution is decolorized, dilute to a liter with water. It is convenient to place the fuchsine solution on a scale pan and then pass in a current of sulfur dioxide until the weight has increased by 20 grams.

2. Aldehyde-free alcohol. Commercial 95 per cent alcohol is first treated with silver oxide as described by Dunlap,<sup>2</sup> and the distillate then treated with meta-phenylenediamine hydrochloride according to Woodman and Lyford. The procedure is as follows: "Dissolve 1.5 grams of silver nitrate in about 3 cc. of water and add it to a liter of 95 per cent alcohol in a glass-stoppered cylinder, then mix thoroughly. Dissolve 3 grams of potassium hydroxide [purified by alcohol] in 10 to 15 cc. of warm alcohol and, after cooling, pour it slowly into the alcoholic silver nitrate solution, but do not shake. The silver oxide is precipitated in a very finely divided condition and slowly distributes itself throughout the contents of the cyl-

<sup>1</sup> A. G. Woodman and E. F. Lyford, *J. Am. Chem. Soc.*, **30**, 1607 (1908).

<sup>2</sup> *J. Am. Chem. Soc.*, **28**, 395 (1906).

inder. Let stand quietly overnight or until the precipitated silver oxide has completely settled. Either siphon off the clear supernatant liquid from the sediment or filter, and then distil" (Dunlap). "To the distillate is added 25 grams of meta-phenylenediamine hydrochloride per liter and a fairly rapid current of air drawn through the solution for three hours. The alcohol is then distilled, the first 100 cc. being rejected. The remainder of the distillate should give absolutely no color when tested for aldehyde in the manner described under the estimation of benzaldehyde. It will remain suitable for use for several weeks at least, if kept cold and in the dark, preferably in an ice-chest." (Woodman and Lyford.)

3. Standard benzaldehyde solution. Make a solution of freshly distilled benzaldehyde in aldehyde-free alcohol so that each cubic centimeter contains 1 mg. of benzaldehyde. This dilute solution keeps well.

**Procedure.**—Two cubic centimeters of the sample (which should contain 5 to 10 mg. of benzaldehyde) are placed in a colorimeter tube and made up to 20 cc. with aldehyde-free alcohol. Several standard solutions are made up containing 2, 4, 6, etc., mg. of benzaldehyde and placed in similar colorimeter tubes. All tubes are then placed in a water-bath at 15° C. and allowed to stand until the solutions reach the temperature of the bath. Have ready Schiff's reagent, also at 15°, and add 20 cc. of it to each tube as rapidly as possible, thoroughly mix, allow the tubes to stand 10 minutes in the bath, and match the sample tube against a standard. The matching must be made as quickly as possible, since the color rapidly deepens if the temperature rises much above 15°.

#### Notes.

1. Pure fuchsin must be used in order to obtain a colorless solution. The solution can be relied upon for about 10 days. The sulfuric acid formed by the oxidation of the sulfur dioxide decreases the sensitiveness of the solution, so that by the end of 10 days a fresh solution should be prepared.

2. Within reasonable limits the color is approximately proportional to the depth of the solution and the concentration of the benzaldehyde; but, as a matter of convenience in reading, it is best to have the concentrations of the sample and standards within 5 to 10 mg. per 40 cc.

**DETERMINATION OF CITRAL BY FUCHSINE-SULFUROUS ACID**

This method has been adapted to the determination of citral<sup>3</sup> (geranial) in lemon oils and extracts.

**Reagents.**

1. Aldehyde-free alcohol. Prepare according to directions on page 412.

2. Fuchsine-sulfurous acid.—Dissolve 0.5 gram of fuchsine in 100 cc. of water and add a solution of sulfurous acid containing 16 grams of sulfur dioxide. Let stand until colorless and dilute to a liter. Prepare fresh every 2 or 3 days.

3. Standard citral solution. One gram of pure citral is dissolved and made up to a liter with 50 per cent (by volume) alcohol (aldehyde-free).

**Procedure.**—Use a 2-gram sample for lemon oil and 20 to 30 grams in the case of lemon extracts. Place the sample in a 100 cc. volumetric flask, dilute to the mark, and mix thoroughly. Allow the solution to cool to 15° in a water-bath and transfer about 4 cc. of it to a colorimeter tube. Then add to the tube 20 cc. of aldehyde-free alcohol, 20 cc. of fuchsine-sulfurous acid solution, and dilute to 50 cc. with alcohol. Mix and allow the solution to come to 15° in the water-bath. Prepare a set of standards similarly. Make color comparison at 15°. The concentration of the sample and comparison solution must be approximately the same when a colorimeter is used, otherwise, a correction must be made. To avoid making a correction, use two samples; the first one to determine the approximate amount of citral present.

**Note.**—The color developed by lemon oil is not quite the same shade as that developed by pure citral. This may be due to the presence of citronellal, since limonene does not affect the color of the standard.

<sup>3</sup> E. M. Chace, J. Am. Chem. Soc., **28**, 1472 (1906).

## CHAPTER XL

### DYES

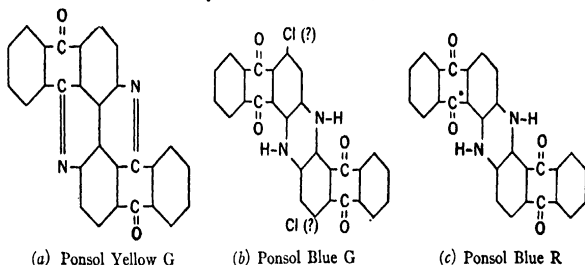
#### DETERMINATION OF CERTAIN VAT DYES BY REDUCTION WITH ALKALINE SODIUM HYPOSULFITE

##### METHOD OF YOE AND EDGAR

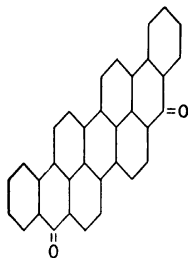
DYES of the indanthrene series are usually applied to the fabric in the reduced condition, reduction being effected by an alkaline solution of sodium hyposulfite. Although the most favorable conditions for practical use of these dyes are very well known, relatively little seems to have been established as to the mechanism by which the reduction occurs, and the influence of different factors upon the rate of the reaction. In order to make such a study it is necessary to determine accurately the amount of reduced dye at frequent intervals (5 or 10 minutes).

In a recent study of this problem<sup>1</sup> a colorimetric method was employed which consisted in comparing the reduced dye solution against a standard made by reducing completely a known weight of dye, or comparing with a standardized colored glass.

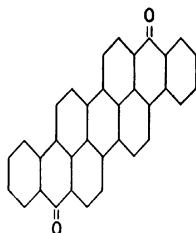
The dyes studied were furnished by E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware, and were known by them as Ponsol Yellow G, Ponsol Blue G, Ponsol Blue R, Ponsol Dark Blue BR, Ponsol Violet RR. They have the following formulas:



<sup>1</sup> J. H. Yoe and G. Edgar, *J. Phys. Chem.*, **27**, 65 (1923); J. H. Yoe, *ibid.*, **28**, 1211 (1924). Note—In the first reference  $\text{NaHSO}_2$  should read  $\text{Na}_2\text{S}_2\text{O}_4$ .



(d) Ponsol Dark Blue BR



(e) Ponsol Violet RR

### Reagents.

1. Sodium hydroxide. The strength that gives best results must be determined for each dye and for a given set of conditions. In the above work 0.5 N NaOH was found best for Ponsol Yellow G and 0.25 N NaOH best for Ponsol Blue G.

2. Sodium hyposulfite ("hydrosulfite"). Use the solid salt. Provided a sufficient amount is present, the concentration of the  $\text{Na}_2\text{S}_2\text{O}_4$  seems to have little effect upon the reduction and solution of the dye.

3. Hydrogen. Use stick zinc and sulfuric acid in any convenient type of generator. Wash the hydrogen by passing it first through a bottle containing sodium hydroxide solution and then through one containing alkaline pyrogallol solution.

4. Standard dye solution. On account of rapid atmospheric oxidation, the standard must be prepared along with the sample and color comparison must be made at once. A thin layer of a colorless mineral oil placed on top of the reduced solutions in the colorimeter tubes will retard the oxidizing action of the air.

5. Standard colored glasses. On account of the rapid atmospheric oxidation of the reduced dye solution, it may be more convenient to standardize a series of colored glasses corresponding to different intensities of the reduced dye. These glasses must, of course, be standardized and used under the same conditions as employed in the treatment of the dye sample. Care must be taken to use only glasses having the identical *tint* of the reduced dye solution and each glass should be marked in such a way that its position in the colorimeter is always the same, thus avoiding an error due to any irregularities in the glass.

**Procedure.**—Place a weighed sample of the dye in a beaker (tall form and without lip) fitted with a wooden cover provided with a hole through which the stirrer (glass) passes and others through which hydrogen can be introduced and samples for analysis withdrawn. The tip of the inlet hydrogen tube is adjusted to within 1 cm. of the surface of the vat solution. The air in the beaker is then replaced by hydrogen, the stirrer (conveniently motor-driven) set in motion, and a measured volume of a solution of NaOH and  $\text{Na}_2\text{S}_2\text{O}_4$  is added. Stirring is continued until reduction is complete, a current of hydrogen being passed continuously into the beaker. When reduction is complete an aliquot of the solution is removed by means of a pipette, run into a colorimeter tube and its color compared at once. If the color is too intense for comparison, dilute the aliquot to a definite volume with a solution of NaOH and  $\text{Na}_2\text{S}_2\text{O}_4$  and place a measured volume of the diluted solution in the comparison tube.

#### Notes.

1. The above procedure should serve only as a suggested outline, the details of which must be worked out for any particular dye. In the work of Yoe and Edgar, and of Yoe (*loc. cit.*), for example, it was found that the color of the reduced dye solution was exactly matched to the eye by cobalt glass. Hence, it was possible to make a series of permanent standards out of cobalt glass.

2. The results of the investigations by Yoe and Edgar, and by Yoe (*loc. cit.*), indicate that the reaction between the dye and the alkaline hyposulfite is very rapid, that an insoluble crystalline reduced dye (dark blue) is first formed, and that the latter is then peptized by hydroxyl ions to form a dark blue sol. The *rate* of peptization, and the *amount of dye stuff* peptized by a given solution, depend upon the state of subdivision of the dye. The rate of reduction is much faster than the rate of peptization. The sol is coagulated by positive ions.



## CHAPTER XLI

### FORMALDEHYDE AND FURFURAL

#### DETERMINATION OF FORMALDEHYDE BY MORPHINE SULFATE

If formaldehyde vapor is allowed to come into contact with a freshly prepared sulfuric acid solution of morphine, a pink to dark blue color is produced, according to the amount of formaldehyde present.<sup>1</sup>

**Reagent.**—Morphine sulfate solution. Dissolve 0.35 gram of white, crystalline morphine sulfate in 100 cc. of cold sulfuric acid, sp. gr. 1.84. This solution must be prepared fresh as needed, since the sulfuric acid slowly decomposes the morphine, even at room temperature.

**Procedure.**—A suitable volume of the test solution (say 60 cc.) is placed in a 3-in. crystallizing dish, 1 cc. of the morphine sulfate solution is floated upon the surface of the test solution by means of a 1-in. watch-glass, and the dish immediately covered with a glass plate. Keep the dish and its contents at about 20°. Compare the color produced in the morphine sulfate solution with those produced by known amounts of formaldehyde in a series of standards prepared along with the test solution, care being taken to start all tests at the same time and under like conditions as to quantity of reagent, size and shape of vessels, temperature, etc.

#### Notes.

1. The above method will detect 4 parts of formaldehyde in 1,000,-000 parts of solution. About 2½ hours are required for the faint coloration to develop.

2. By noting the time required for the first ring or color to appear, an approximate idea may be obtained as to the quantity of formaldehyde present. Using a standard solution of milk, containing a known amount of formaldehyde, a series of solutions was prepared and tested.

<sup>1</sup> F. Bonnet, Jr., *J. Am. Chem. Soc.*, **27**, 601 (1905).

The time to the appearance of color was noted. The following results were obtained by Bonnet:<sup>2</sup>

TABLE XXXVIII

TEMPERATURE 20° C.

Number	Containing HCHO	Remarks
1	Pure milk	No coloration after 3 hours.
2	4 : 100	Coloration appeared in a few minutes, which soon turned black.
3	4 : 1000	Coloration appeared in a few minutes. Upon standing it also turned black.
4	8 : 10,000	Good ring coloration after about 8 minutes.
5	4 : 10,000	Good ring coloration after about 15 minutes.
6	8 : 100,000	Fairly good ring coloration in about 45 minutes.
7	4 : 1,000,000	Fairly good ring coloration in about 1 hour.
8	8 : 1,000,000	Slight coloration throughout, in about 1½ hours.
9	4 : 1,000,000	Slight coloration throughout, in about 2½ hours.

DETERMINATION OF FURFURAL BY ORCINOL <sup>3</sup>

This method is based upon the blue color produced by heating a solution containing a small quantity of furfural to which has been added a dilute solution of orcinol containing acetic acid and concentrated hydrochloric acid containing a little ferric chloride. By this method as little as 4 mg. of furfural per liter may be detected.

## Reagents.

1. Ferri-hydrochloric acid reagent. Add about 60 mg. of ferric chloride,  $\text{FeCl}_3$ , to a liter of hydrochloric acid, sp. gr. 1.19.

2. Orcinol-acetic acid solution. Dissolve 1 gram of orcinol in 1600 cc. of pure acetic acid. The acetic acid must, of course, be free from furfural.

3. Standard furfural solution. Dissolve 1 gram of furfural in 10 cc. of pure acetic acid, add distilled water sufficient to make a liter of solution, and then mix thoroughly. One cubic centimeter of this solution contains 1 mg. of furfural. The solution keeps well.

<sup>2</sup> *Loc. cit.*<sup>3</sup> P. Fleury and G. Poirot, *J. pharm. chim.*, **26**, 87 (1922); *Bull. soc. chim. biol.*, **4**, 252 (1922).

For the standard comparison solution, dilute 10 cc. of the above solution with 1 cc. of pure acetic acid and water sufficient to make 100 cc. Mix thoroughly. This solution contains 0.1 mg. furfural per cubic centimeter.

**Procedure.**—Place 1 cc. of the dilute furfural solution (0.1 mg. furfural) in a test tube. In a second test tube put 1 cc. of the sample containing approximately 0.1 mg. of furfural. To each tube add simultaneously the following reagents: 4 cc. of the orcinol-acetic acid solution and 5 cc. of the ferri-hydrochloric acid reagent. Place the tubes in a water-bath, heat them for exactly 1 minute, remove the tubes and allow them to stand 30 minutes. The bluish-green color, at first produced, turns blue on standing and reaches a maximum intensity after 30 minutes. It remains unchanged for 30 to 40 minutes more and then fades. As soon as the solutions have stood 30 minutes, they are transferred to the colorimeter and matched.

#### Notes.

1. The amount of furfural in the sample taken for comparison should be such that the height of liquid in the tube containing the sample will not vary more than 20 per cent from the height of the liquid in the tube containing the standard.

2. The following results obtained by Fleury and Poirot<sup>4</sup> illustrate the accuracy and precision of the method:

MILLIGRAMS OF FURFURAL PER LITER

Present	Found
110.0	{ 111.11 110.13
190.0	{ 189.04 188.32
440.0	{ 444.0 444.0

3. The method of dilution cannot be used.

4. The orcinol-acetic acid solution and the standard furfural solu-

<sup>4</sup> *Loc. cit.*

tion are quite stable. Solutions 6 months old were still satisfactory. (Fleury and Poirot, *loc. cit.*)

#### DETERMINATION OF FURFURAL BY ANILINE<sup>5</sup>

##### Reagents.

1. Hydrochloric acid, sp. gr. 1.125.
2. Colorless aniline. Redistill ordinary dark-colored aniline. Keep in the dark.
3. Furfural-free alcohol. Commercial 95 per cent alcohol redistilled over caustic soda or potash is practically free from furfural.
4. Standard furfural solution. Dissolve 1 gram of redistilled furfural in 100 cc. of 95 per cent (by volume) alcohol. This solution keeps well. Standards are prepared by diluting 1 cc. of the strong solution to 100 cc. with 50 per cent (by volume) alcohol. One cubic centimeter of the dilute solution contains 0.1 mg. of furfural.

**Procedure.**—A measured volume (10 or 15 cc.) of the sample containing a few tenths of a milligram of furfural is placed in a colorimeter tube, diluted to 50 cc. with 50 per cent (by volume) alcohol free from furfural, and the solution brought to 15° in a water-bath. Two cubic centimeters of aniline and 0.5 cc. of hydrochloric acid, sp. gr. 1.125, are then added and the solution thoroughly mixed. After standing in the bath at 15° for 15 minutes the tube is removed and the color of the solution compared against standard furfural solutions similarly prepared. The standard which most nearly matches the color in the sample is used in the colorimeter. The intensity of the color is directly proportional to the amount of furfural present, so that calculation can be made directly from the colorimeter reading.

##### Notes .

1. The temperature must be carefully regulated since it greatly affects the reaction. The sample and standard solutions are compared at 15°, which should be the approximate temperature of the room.

2. Hydrochloric acid was used by Tolman<sup>6</sup> instead of acetic acid because the acetic acid available gave a "very decided color reaction, which interfered with the test." Acetic acid has been found by a

<sup>5</sup> L. M. Tolman, J. Am. Chem. Soc., **28**, 1629 (1906).

<sup>6</sup> *Loc. cit.*

number of observers to give a color reaction with Schiff's reagent. Another advantage hydrochloric acid has over acetic acid is its more uniform quality—a necessity for comparable results.

3. "In order to settle the question whether all the furfural will be found in the distillate and also whether there is any formation of furfural in distillation, the determination was made on a number of samples of slightly colored and colorless spirits, in the original and in the distillate. The results on 120 samples gave an average of 23.4 mg. per liter on the original and 23.2 mg. per liter on the distillate. This shows that there is no loss or gain of furfural by distillation; it also shows that the method is reliable, as the same conditions held in these determinations as in the work on aldehydes."<sup>7</sup>

<sup>7</sup> Tolman, *loc. cit.*

## CHAPTER XLII

### ORGANIC SUBSTANCES (MISCELLANEOUS) AND PENTOSE

#### DETERMINATION OF ORGANIC SUBSTANCES BY POTASSIUM BICHROMATE-SULFURIC ACID

##### METHOD OF HEIDENHAIN<sup>1</sup>

A DILUTE solution of pure potassium bichromate in pure concentrated sulfuric acid may be boiled for a long time with little or no decomposition of the chromic acid. If, however, organic matter is present the chromic acid is reduced, the organic matter being oxidized quantitatively or nearly so. The solution turns different shades of color ranging from a pure green (complete reduction of chromic acid) to pure orange (undecomposed chromic acid) with all shades of yellowish green to greenish yellow between these extremes. The method has been used successfully in the estimation of carbohydrates in the wash liquors from washing bone-black after its decolorizing power had been exhausted, for the estimation of glycerol in soap lyes, for the determination of tartrate in baking powders, and for the estimation of alcohol in vinegars.

**Preparation of the Colorimetric Standards.**—The oxidation of organic substances by chromic acid in presence of sulfuric acid is only complete, or nearly so, when an excess of the chromic acid is employed. As this excess imparts to the solution a yellowish tint, we should exclude the purer green tints from our scale of colors if we always work with an excess of the chromate. As it happens, the tints in which the green predominates are more easily discerned than those in which the orange predominates. In order not to lose this advantage and also to make the range of colors as wide as possible, Heidenhain sacrificed the feature of the quantitative reaction in the preparation of the colorimetric standards, and based the scale of colors on purely empirical results. The color produced by equivalent quantities of the bichromate and a substance is called 100; the color produced by the same amount of

<sup>1</sup> J. Ind. Eng. Chem., 11, 297 (1919).

bichromate and 90 per cent of the equivalent quantity of the substance is called 90; and so on to zero, the color of the pure undecomposed bichromate.

**Procedure.**—Prepare a 0.2 N  $K_2Cr_2O_7$  solution by dissolving 9.8067 grams in water and diluting to a liter. Further, prepare a 0.2 N solution of the organic substance to be determined, calculating its quantity from the number of equivalents of oxygen required for the complete oxidation of the molecule. For instance, in the case of dextrose,  $C_6H_{12}O_6$ , 12 atoms of oxygen are required for complete oxidation.

Therefore,  $\frac{180}{12 \times 2 \times 5} = 1.5$  grams is the proper quantity to make 1000 cc. of a 0.2 N solution.

Select a number of glass-stoppered bottles of cylindrical shape and uniform diameter, and a capacity of about 100 cc. (4 oz. oil sample bottles answer fairly well. Rubber stoppers may be used if care is taken that the contents of the bottles do not come in contact with them later). Put 80 cc. of water into the bottles and see whether the water stands equally high in them. If satisfactory in this respect,

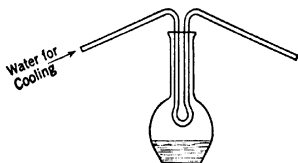


FIG. 53.

make a mark with the file to indicate the 80 cc. volume.

Select a 300 cc. flask with long and narrow neck and make a reflux condenser, by bending a glass tube into the shape shown in Fig. 53, to fit the neck of the flask.

Now transfer into the flask 25 cc. of the 0.2 N bichromate solution and 25 cc. of the 0.2 N solution of the substance, mix, and add 30 cc. of conc. sulfuric acid in a small stream while the contents of the flask are rotated. By the addition of the acid the temperature of the liquid is raised close to the boiling-point. Put the flask without delay under the reflux condenser, heat quickly to boiling, and keep at the boiling-point for exactly 5 minutes, regulating the flame so that the liquid boils up from time to time. Cool and transfer the liquid to one of the cylindrical bottles. Fill to the 80 cc. mark with water, mix, and put a label on the neck of the bottle with the mark 100 as the first standard in the scale of colors.

Prepare the second standard by mixing 25 cc. of 0.2 N bichromate solution with 22.5 cc. of the 0.2 N solution of the substance and 2.5 cc.

of water, adding 30 cc. of sulfuric acid and proceeding further as described above. Mark this one 90.

TABLE XXXIX

0.2 N $K_2Cr_2O_7$ Solution, Cubic Centimeters	0.2 N Solution of Substance, Cubic Centimeters	Water, Cubic Centimeter	Sulfuric Acid, Cubic Centimeter	Color Indicates that Substance is Present in Concentration of
25	25.0	0.0	30	100% of 0.2 N
25	22.5	2.5	30	90% of 0.2 N
25	20.0	5.0	30	80% of 0.2 N
25	17.5	7.5	30	70% of 0.2 N
25	15.0	10.0	30	60% of 0.2 N
25	12.5	12.5	30	50% of 0.2 N
25	10.0	15.0	30	40% of 0.2 N
25	7.5	17.5	30	30% of 0.2 N
25	5.0	20.0	30	20% of 0.2 N
25	2.5	22.5	30	10% of 0.2 N
25	0.0	25.0	30	0% of 0.2 N

Prepare the other standards by always using 25 cc. of 0.2 N bichromate solution and 30 cc. of sulfuric acid, but varying the volume of the 0.2 N solution of the substance and the water according to Table XXXIX.

#### Notes.

1. Standards prepared with substances which are completely or almost completely oxidized have been found to be stable for several months.

2. The oxidimetric equivalents of a few substances of common occurrence are given in Table XL.

The method may also be used for substances, the oxidation of which does not lead to definite products. However, whether or not permanent standards may be obtained with such substances is a matter of experimentation. For such cases, Heidenhain recommends making comparisons with a set of permanent standards, the relative values of which are easily determined and tabulated for ready use.



TABLE XL

Substance	1000 Cc. of a 0.2 N Solution Contains, Grams	Products of Oxidation
Dextrose.....	1.500	CO <sub>2</sub> ,H <sub>2</sub> O
Dextrin.....	1.350	CO <sub>2</sub> ,H <sub>2</sub> O
Maltose.....	1.425	CO <sub>2</sub> ,H <sub>2</sub> O
Tartaric acid.....	3.000	CO <sub>2</sub> ,H <sub>2</sub> O
Oxalic acid.....	12.600	CO <sub>2</sub> ,H <sub>2</sub> O
Formic acid.....	4.600	CO <sub>2</sub> ,H <sub>2</sub> O
Glycerol.....	1.314	CO <sub>2</sub> ,H <sub>2</sub> O
Ethyl alcohol.....	2.300	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>

Acetic acid is not oxidized.

3. Substances to be tested may be dissolved in alkalis, ammonia, sulfuric and acetic acids. Hydrochloric and nitric acids interfere with the determination.

4. The above method may also be used for the approximate determination of inorganic substances having reducing properties. For example, Heidenhain has recently used it to analyze lime-sulfur solutions.<sup>2</sup> The total reduction of the lime-sulfur solution agreed with the result obtained by the methods for the determination of sulfur in the different forms in which it is present in this preparation. In this determination, the method is slightly modified as follows: A larger excess of dichromate (about double the theoretical quantity) is used, together with an equal volume of sulfuric acid. To this mixture is added the diluted lime-sulfur solution, the tip of the pipette being held below the surface of the liquid. Heating for about half a minute completes the oxidation of the sulfur.

#### DETERMINATION OF PENTOSE IN FREE OR COMBINED FORM

Upon steam distillation of a reaction mixture containing hydrochloric acid of 12 to 20 per cent concentration, a complete hydrolysis of a pentose-containing substance is obtained and the furfural thus formed is transferred quantitatively, provided certain precautions are taken. (See Notes 1, 2 and 3.) When the furfural solution is mixed with aniline and acetic acid a red color is formed. This color

<sup>2</sup> Private communication from Mr. H. Heidenhain.

reaction will detect exceedingly small concentrations of furfural; and solutions that contain 0.00005 per cent or more of furfural can be quantitatively compared in a colorimeter. The method described below is that of Hoffman<sup>3</sup> and is based upon the methods of Pervier and Gortner<sup>4</sup> and of Youngburg and Pucher.<sup>5</sup>

#### Reagents.

1. Hydrochloric acid, 20 per cent.
2. Glacial acetic acid.
3. Sodium hydroxide, 10 per cent.
4. Aniline. Use only freshly distilled aniline.
5. Standard furfural solution. Prepare from furfural which has been repeatedly distilled under reduced pressure. Always make up with toluene-saturated water to prevent bacterial decomposition.

**Apparatus and Procedure.**—The apparatus used for the conversion of pentose into furfural is the ordinary steam distillation device, except that the distilling flask has no rubber stoppers. Instead, the side arm of the flask is made quite long so as to serve as the inner tube of a condenser, and has attached over it the jacket of a condenser. The tube that runs from the steam generator into the distilling flask is sealed by mercury with the flask. A 500 cc. distilling flask is most convenient to hold the reaction mixture. The speed of the steam production may be minimized by using a small flask as a generator. Distilled water containing potassium permanganate and sodium hydroxide is used as a source of steam.

Steam distillation of furfural itself is complete within half an hour, but 3 hours are required for complete production of furfural from xylose and from ribose compounds, in order to convert the pentose into furfural. Fifty cubic centimeters of 20 per cent hydrochloric acid are used.

The distillate is collected in a volumetric flask holding a little more than the amount of liquid expected. The distillate is then titrated in this flask with 10 per cent sodium hydroxide from a burette until neutral to phenolphthalein. The solution is then diluted to the mark and thoroughly mixed. The standard solution equivalent to the amount of furfural distilled is placed in a flask of the same size and

<sup>3</sup> J. Biol. Chem., **73**, 15 (1927).

<sup>4</sup> Ind. Eng. Chem., **15**, 1167 (1923).

<sup>5</sup> J. Biol. Chem., **61**, 741 (1924).

treated with hydrochloric acid and sodium hydroxide until the solution is neutral to phenolphthalein and the sodium hydroxide added equals the amount added to the unknown. This solution is also diluted to the mark and thoroughly mixed. The two solutions now contain the same concentration of sodium chloride and are exactly neutral to phenolphthalein. Six cubic centimeters of each solution are transferred to a test tube; each is treated with 0.5 cc. of aniline and 4.0 cc. of glacial acetic acid, allowed to stand for 10 to 15 minutes in the dark, and then compared in a colorimeter.

#### Notes.

1. The water used as the source of steam contains a little potassium permanganate and sodium hydroxide to prevent the distillation of chlorine or other volatile substances.

2. Pervier and Gortner have shown that the amount of distillate is unimportant, a slow stream of steam being just as effective as a fast one. The temperature of the reaction mixture, however, is important. It should be kept, as Pervier and Gortner say, at 103–105°; for, at higher temperatures, decomposition may occur, and besides the hydrochloric acid is soon distilled out, and at lower temperatures the volume in the distilling flask increases, producing a diminution of the concentration of the hydrochloric acid and a consequently decreased speed of reaction. This constancy of temperature can be acquired with a little practice without the use of a thermometer, by regulating the size of the flame under the distilling flask. Good results are obtained if the volume is allowed to remain constant or to decrease very slowly. In 3 hours, under such conditions, the concentration of hydrochloric acid never gets below 12 per cent.

3. Furfural must not come in contact with rubber stoppers. This condition is made conveniently possible by the use of a distilling flask with a mercury seal and with a long side arm serving as a condenser.

4. Hoffman<sup>6</sup> has shown that the above method gives theoretical results in 3-hour distillations using 50 cc. of 20 per cent hydrochloric acid in the case of xylose, adenosine, guanosine, adenine, nucleotide, and guanidine nucleotide. Arabinose is only slowly converted into furfural, and the pyrimidine nucleotides are only very slowly hydrolyzed. Yeast nucleic acid gives up a little more than half its pentose in 3 hours, as is to be expected.

<sup>6</sup> *Loc. cit.*

## CHAPTER XLIII

### PHENOL AND SALICYLIC ACID

#### DETERMINATION OF PHENOLS BY THE FOLIN AND DENIS REAGENT

##### METHOD OF VORCE<sup>1</sup>

THIS method was developed by Vorce for the determination of minute amounts of phenols in polluted natural waters.

##### Reagents.

1. Citric acid, crystallized.
2. Sodium hydroxide, solid.
3. Sodium carbonate, saturated solution.
4. Hydrogen peroxide, free of stabilizers containing a benzene nucleus.

5. Folin and Denis phenol reagent.<sup>2</sup> The phenol reagent is prepared as follows: To 750 cc. of distilled water add 100 grams of sodium tungstate, 18 grams of molybdenum trioxide, and 50 cc. of 85 per cent phosphoric acid ( $\text{H}_3\text{PO}_4$ ), and boil under the reflux condenser for 2 hours. Cool and dilute to 1 liter. The standard phenol solution contains 1 mg. of phenol to 1 cc. of solution and is preserved in a black, paper-covered, glass-stoppered bottle with the stopper kept sealed with wax and the bottle kept away from light as much as possible. Thus preserved, the solution will keep at least 6 months without change of strength.

**Procedure.**—(a) *Preparation of Sample.*—Measure accurately 2 or 3 liters of the sample into a flask, add 2 to 4 grams of solid caustic soda, and shake frequently until dissolved and the precipitated bases and coagulated albuminous matter have separated. Filter into a similar flask, and wash flask and filter once with water. To the filtrate in the second flask add 25 cc. of peroxide of hydrogen ( $\text{H}_2\text{O}_2$ ), being

<sup>1</sup> Ind. Eng. Chem., **17**, 751 (1925).

<sup>2</sup> J. Biol. Chem., **12**, 239 (1912).

particular to use a kind stabilized with a compound that does not contain a benzene nucleus. Shake thoroughly and allow to stand overnight, uncorked, to allow as much as possible of the excess peroxide to escape. Fit the flask with a rubber stopper carrying a constant-level delivery tube. Place at least 20 grams of solid sodium hydroxide in a 15 cm. nickel or porcelain evaporating dish, and if 1 liter of the sample was taken support on a stand over a Bunsen burner. If 2 or more liters of the sample were taken, place the dish on a constant-level water-bath and run overnight. Support the inverted flask over the dish so that the level of the water in the dish will maintain a nearly constant volume of not more than 200 cc. Adjust the burner (case of 1 liter sample) so that the contents of the dish are just below the boiling-point. When the flask is empty, rinse it and the delivery tube once or twice with water and continue evaporation until the contents of the dish do not exceed 200 cc.

Transfer the concentrated solution to a long-necked, 750 cc. Kjeldahl flask, and chill in ice or cold tap water. Add to the flask, all at once, 45 grams of crystallized citric acid and, when dissolved, test with litmus paper to insure acidity. Fit the neck of the flask with a rubber stopper carrying a return-flow bulb trap with vapor outlet tube bent downward to fit the top of the vertical condenser, and a glass stopcock separatory funnel of 50 to 75 cc. capacity. Any form of condenser that will deliver the distillate thoroughly cold will suffice. If the projecting bottom end of the condenser tube is not long enough to reach the bottom of an ordinary 500 cc. volumetric flask, extend it with a rubber sleeve and glass tube. Connect the distilling flask to the condenser and place below, on sufficient blocking, a 500 cc. volumetric flask receiver, first adding a few cubic centimeters of water to seal the end of the condenser tube. Heat the distillation flask by a direct flame and distill until about 125 cc. have passed over, when the contents of the distilling flask will be almost entirely sodium citrate dissolved in its crystal water.

Lower the receiver until the tip of the condenser tube is again just sealed by the distillate, and without removing the burner introduce through a stopcock funnel 100 cc. of distilled water. Continue distillation and repeat this procedure each time 100 cc. have distilled over until the receiving flask is nearly filled to mark. After allowing the distillate to reach room temperature, make up to 500 cc. with distilled water and mix thoroughly. This solution now contains all but

traces of the phenol in the sample originally measured into the reservoir flask of the concentrating apparatus.

(b) *Color Comparison*.—For the color comparison standards, some of the strong standard is diluted to a 10 p.p.m. solution for use in making up the color standard tubes; this 10 p.p.m. solution should be freshly prepared each time as it does not keep well. Vorce uses 50 cc. Nessler tubes for the comparison, and in making the color standards, in 50 cc. tubes, 1 cc. of the Folin and Denis phenol reagent should be added for each 1 p.p.m. of phenol in the standard, and followed by 5 cc. of saturated sodium carbonate solution. At least an hour is required for full development of color in tubes of less than 4 p.p.m. of phenol, and with more than 4 p.p.m. of phenol the sample must be diluted for comparison. Tubes are best viewed from the side against a white background, by reflected light. When the color standards have fully developed, the sample tube, prepared at the same time as the standards, is compared with them and the indicated content noted. If this indicated content of phenol in parts per million varies from the number of cubic centimeters of phenol reagent used in preparing the sample tube, make up two more tubes of the sample distillate, using in one as many cubic centimeters of phenol reagent as the parts per million of phenol indicated by the trial tube, and in the other, 0.5 cc. more than the indication. These two tubes will almost always give so nearly the same color that they cannot be distinguished from each other. If either should be visibly darker, compare the darker one with the color standards and take the reading as the phenol content of the distillate. Calculation to original sample is obvious.

#### DETERMINATION OF PHENOL BY MILLON'S REAGENT

This method depends upon the yellow to red coloration produced by the addition of Millon's reagent to a solution of phenol containing nitric acid.<sup>3</sup>

##### Reagents.

1. Nitric acid, sp. gr. 1.42.
2. Millon's reagent. Dissolve 400 grams (30 cc.) of mercury in 570 cc. of nitric acid, sp. gr. 1.42, and dilute the solution with two volumes of water.

<sup>3</sup> H. Bach, Z. anal. Chem., 50, 736 (1911).

3. Phenol. Prepare a water solution of pure phenol containing 0.1 mg. of phenol per cubic centimeter.

**Procedure.**—Place 10 cc. of the test solution (containing between 0.3 and 1.5 mg. of phenol) in a comparison cylinder of narrow-bore (1 cm. diam.), add 0.2 cc. of Millon's reagent and 3 drops (0.1 cc.) of nitric acid, sp. gr. 1.42, and heat in a hot water-bath almost to boiling. Allow the solution to stand 2 hours and match its color against a standard solution prepared similarly and along with the test solution.

#### Notes.

1. By heating the solutions to the boiling-point the color is rendered sufficiently stable for colorimetric comparison.

2. If the color of the treated test solution is pale yellow to bright rose, the phenol content is between 0.2 and 0.7 mg. A bright rose to red indicates 0.8 to 1.8 mg. of phenol, and red to deep red indicates 1.8 to 3 mg. of phenol.

3. Best results are obtained when the phenol concentration is between 30 and 150 mg. per liter. If the sample gives only a very pale yellow color upon treatment, take a 100 cc. portion of it, acidify with 5 cc. of conc. nitric acid, distill over 20 cc., and use 10 cc. of the distillate for the test.

#### DETERMINATION OF SALICYLIC ACID BY IRON ALUM

This method is adapted to the estimation of salicylic acid in food.

#### Reagents.

1. Sulfuric acid, 6 N.
2. Sodium hydroxide, 6 N.
3. Ether.
4. Iron alum, 1 per cent. Add a drop or two of sulfuric acid per each 100 cc. of solution.

**Procedure.**—The salicylic acid is extracted with ether and the ethereal solution in turn shaken with two successive portions of water containing a few drops of sodium hydroxide solution. Carefully neutralize the aqueous extract and dilute to 250 cc. Place an aliquot volume in a colorimeter tube or a Nessler cylinder, add 1 or 2 cc. of 1 per

cent iron alum solution, mix, and compare the color with that given by a known quantity of salicylic acid similarly treated.

**Note.**—According to Harvey,<sup>4</sup> 1 part of salicylic acid in 3,000,000 parts of water can be detected.

#### DETERMINATION OF SALICYLIC ACID BY FEHLING'S SOLUTION \*

This determination is similar to the salicylic acid method for the determination of copper, which see. The sample is treated with Fehling's solution and matched against a series of standards prepared at the same time.

##### Reagents.

1. Acetic acid, 10 per cent.
2. Potassium nitrate, 2 per cent.
3. Fehling's solution. *Solution A.*—Dissolve 34.64 grams of pure copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , in 500 cc. of water. *Solution B.*—Dissolve 60 grams of sodium hydroxide and 173 grams of Rochelle salt (sodium potassium tartrate,  $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ) in 500 cc. of water. Mix equal volumes of Solutions *A* and *B* and use at once.
4. Standard salicylic acid solution. Dissolve 0.1000 gram of pure salicylic acid, or 0.1159 gram of pure sodium salicylate, in water and dilute to a liter. Thoroughly mix. One cubic centimeter of the solution contains 0.1 mg. of salicylic acid.

**Procedure.**—The sample should be adjusted to contain between 0.01 and 0.1 mg. of salicylic acid. It is concentrated to 2 or 3 cc. and placed in a small test tube. At the same time have ready a series of similar test tubes containing 0, 0.2, 0.4, 0.6, 0.8, and 1.0 cc. of the standard solution. Add to the sample tube, and to each tube of standard, 2 cc. of a solution of 1 part of Fehling's solution to 10 parts of water, 5 drops of the potassium nitrate solution, 5 drops of the acetic acid solution, and water sufficient to bring the volume up to approximately 5 cc. The volume of the solutions must be the same. The tubes are now placed in boiling water, heated for 45 minutes, and then compared. The comparison must be direct. The method of dilution is not satisfactory.

<sup>4</sup>Analyst, **28**, 2 (1903).

\* F. Schott, Z. Nahr. Genussm., **22**, 727 (1911).



**Notes.**

1. Sucrose, glucose, lactose, invert sugar, and traces of iron do not interfere with the analysis, but free mineral acids, tartaric and citric acids, and large amounts of iron do.
2. Fehling's Solutions *A* and *B* keep perfectly unmixed, but must be used at once after they are mixed.
3. The presence of 0.001 mg. of salicylic acid per cubic centimeter can be detected in a 10 cc. sample. Smaller amounts may be detected and estimated by suitable concentration.

## CHAPTER XLIV

### TANNIN AND THIOPHEN

#### DETERMINATION OF TANNIN<sup>1</sup>

THIS method is based upon the violet color produced when ferrous sulfate reacts with gallotannin in the presence of tartaric acid.

##### Reagents.

1. Ferrous sulfate reagent. Dissolve 1 gram of ferrous ammonium sulfate,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , and 5 grams of Rochelle salt in 20 cc. of water. Prepare fresh as needed.

2. Standard solution. Use either pure pyrogallol or pure gallic acid, preferably the latter. A suitable standard contains 0.1 gram of the pure substance per 100 cc. of water.

**Procedure.**—Place 1 cc. of the standard solution and a measured volume of the tannin solution in Nessler tubes, add to each 2 cc. of the ferrous sulfate reagent, dilute to the 100 cc. mark, mix, and compare the colors both vertically and horizontally. If both colors are too dark, take 50 cc. of each and again compare. Pipette out the darker solution until its color matches the weaker when viewed vertically. Then dilute each solution to 100 cc., mix, and again compare.

##### Notes.

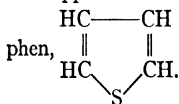
1. The color produced in the above procedure is so stable that the process of dilution and comparison may be repeated several times without recharging the tubes. If very little tannin is present in the unknown solution, it is best to repeat the determination, using a smaller amount of standard.

2. The colorimetric ratio varies with different tannins because the gallotannin content varies. For example, tannin from sumac does not appear to contain any gallotannin and, hence, the colorimetric method fails.

<sup>1</sup> C. A. Mitchell, *Analyst*, 48, 2 (1923).

DETERMINATION OF THIOPHEN IN CRUDE BENZENE<sup>1</sup>

The method is based upon the green coloration produced when benzene containing thiophen is mixed with a sulfuric acid solution of isatin. It is applicable to benzenes containing 0.05 per cent or more of thio-

**Reagents.**

1. Sulfuric acid, sp. gr. 1.84.
2. Isatin-sulfuric acid. Dissolve 0.5 gram of isatin in 1000 grams of sulfuric acid, sp. gr. 1.84.

**Procedure.**—Add 1 cc. of the benzene sample to a mixture of 25 cc. of sulfuric acid and 25 cc. of isatin-sulfuric acid, thoroughly shake, and compare the color with that obtained with pure benzene to which has been added a definite amount of thiophen.

<sup>1</sup> C. Schwalbe, Chem. Ztg., **29**, 895 (1905).

## CHAPTER XLV

### VANILLIN

#### DETERMINATION OF VANILLIN

##### METHOD OF FOLIN AND DENIS<sup>1</sup>

THIS method is based upon the deep blue color obtained when vanillin in acid solution is treated with a phosphotungstic-phosphomolybdic reagent and then sodium carbonate added in excess. Other mono-, di-, and tri-hydric phenol compounds give similar reactions.

The method is short and accurate and requires only 2.5 cc. of extract for from two to four duplicate determinations.

##### Reagents.

1. Sodium carbonate. Use a saturated solution.
2. Lead acetate. Prepare a solution containing 5 per cent basic and 5 per cent neutral lead acetate.
3. Phosphotungstic-phosphomolybdic acid reagent. Add 100 grams of syrupy phosphoric acid (containing 85 per cent  $H_3PO_4$ ) and 700 cc. of water to 100 grams of pure sodium tungstate and 20 grams of phosphomolybdic acid (free from nitrates and ammonium salts), boil for about 2 hours, cool, filter if necessary, and make up to a liter with water.

An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

4. Standard vanillin solution. Dissolve 2.0000 grams of vanillin in 200 cc. of 95 per cent alcohol, dilute to a liter, and thoroughly mix. Dilute 10 cc. of this solution to 200 cc. as needed. Ten cubic centimeters of this solution contain 1 mg. of vanillin.

The strong solution will keep for at least 2 months, but the weaker

<sup>1</sup>O. Folin and W. Denis, *J. Ind. Eng. Chem.*, **4**, 670 (1912).

solution increases, on standing, in its power to produce a blue color when treated with the reagent.<sup>2</sup>

**Procedure.**—Place 5 cc. of the vanilla extract in a 100 cc. volumetric flask, add about 75 cc. of cold tap water and 4 cc. of the lead acetate solution. Dilute to the mark with water and rapidly filter through a folded filter paper. Transfer by means of a pipette 5 cc. of the filtrate to a 50 cc. volumetric flask. In another 50 cc. volumetric flask place 5 cc. of the standard vanillin solution. Add to each flask 5 cc. of the phosphotungstic-phosphomolybdic reagent, allowing the reagent to run down the neck of the flasks so as to wash down any adhering vanillin. Shake the flasks, allow the solutions to stand for 5 minutes, and then dilute to the mark with saturated sodium carbonate solution. Mix thoroughly the contents in each flask and allow to stand 10 minutes. At the end of this time the sodium phosphate will have completely precipitated. Then filter rapidly through a folded filter paper and compare the resulting deep blue solutions in a colorimeter.

If a Duboscq colorimeter is used, "the standard solution is best placed at 20 mm. as experiment has shown that the color produced by the amount of vanillin contained therein (1 mg. in 100 cc.) is most accurately and easily read at this point."<sup>3</sup>

#### Notes.

1. No solution should be used for the colorimetric comparison which is not perfectly clear after filtration. A slight cloudiness of the solution to be read will cut off more light than the standard and, hence, will give a reading too low, with correspondingly high result.

2. Vanillin, even in fairly dilute solution, is precipitated by basic lead acetate, but in the very high dilutions employed in the above procedure no precipitation occurs.

3. Coumarin, extract of tonka bean, and acetanilid do not give a color reaction with the phosphotungstic-phosphomolybdate reagent, and the presence of sugar, caramel, or glycerin does not in any way interfere with or alter the color formation.

4. The table below will give an idea of the agreement between results obtained by the "official method" and those by the colorimetric method. The data are taken from Folin and Denis.<sup>4</sup> The figures

<sup>2</sup> O. E. Harder, *J. Ind. Eng. Chem.*, **5**, 619 (1913).

<sup>3</sup> Folin and Denis, *loc. cit.*

<sup>4</sup> *Loc. cit.*

for the official method were obtained in part by Dr. A. L. Winton and in part by Dr. R. S. Hiltner.

TABLE XLI

Number	Prepare from	Percentage Vanillin by	
		Official method	Colorimetric method
I	Mexican beans, 60 per cent alcohol and glycerin..	0.20	0.20
II	Bourbon beans, 60 per cent alcohol and sugar (U. S. P.).....	0.19	0.19
III *	Mexican and Tahiti beans and maple syrup, prune juice, synthetic vanillin and caramel.....	0.18	0.17
IV †	25 per cent tonka extract, 75 per cent prune juice, 0.15 per cent vanillin and caramel coloring.....	0.15	0.16

\* A. U. S. P. extract.

† An entirely artificial extract.

#### METHOD OF ESTES

When mercuric nitrate in nitric acid solution is added to a solution containing vanillin, a violet to violet-red color is produced. The intensity of the color is directly proportional to the amount of vanillin present. The reaction seems to be characteristic of vanillin and mercuric nitrate (see Note 1) and gives a rapid and accurate method for the estimation of vanillin in extracts.

#### Reagents.

1. Mercuric nitrate reagent. Dissolve metallic mercury in twice its own weight of concentrated nitric acid, sp. gr. 1.42, and then dilute with 25 times its weight of water. These proportions may be varied somewhat, but it is necessary to have a slight excess of nitric acid and the mercury must be in the higher state of oxidation.

2. Standard vanillin solution. Dissolve 1.0000 gram of the purest vanillin in water and dilute to 100 cc. Thoroughly mix. One cubic centimeter of the solution contains 10 mg. of vanillin.

#### ALCOHOLIC EXTRACTS

**Procedure.**—Place 5 cc. of the vanilla extract in a 50 cc. volumetric flask and add 6 cc. of water and 1.5 cc. of the mercuric nitrate

reagent. At the same time place 5 cc. of the standard vanillin solution, 0.5 cc. of the mercuric nitrate reagent and 6 cc. of water in another 50 cc. flask. Set the two flasks in boiling water and heat for 20 minutes. Then remove the flasks, rapidly cool the solutions to room temperature, dilute to the mark, and filter. Compare the solutions in a colorimeter.

#### NON-ALCOHOLIC EXTRACTS

**Procedure.**—The procedure is the same as that for alcoholic extracts, except that 1.0 cc. of the mercuric nitrate reagent is used instead of 1.5 cc. The standard is prepared exactly as directed above for alcoholic extracts.

#### Notes.

1. "This color reaction seems to be characteristic only of vanillin and the acid nitrate of mercury, as acid nitrates of many other metals were prepared in the same way as that of mercury, but in every case they failed to develop a color; also many other organic compounds containing a hydroxyl group and an aldehyde group have been substituted for vanillin in the reaction, but so far no color reaction was found which resembled that of vanillin. Some other organic compounds were found which gave a color reaction with the acid nitrate of mercury reagent; however, the colors produced could never be mistaken for that produced by vanillin."<sup>5</sup>

2. Since the mercuric nitrate reagent, when added to a vanilla extract, causes a precipitation of the resins, coloring matter, etc., it is necessary to add a sufficient quantity of the reagent so as to have an excess to react with the vanillin.

3. The solution is heated after adding the reagent in order to hasten the formation of the maximum intensity of color. The color is produced only slowly at room temperature.

4. The results recorded in the following table were obtained by Estes<sup>6</sup> and represent single determinations, both by the gravimetric

<sup>5</sup> C. Estes, J. Ind. Eng. Chem., 9, 142 (1917).

<sup>6</sup> *Loc. cit.*

and colorimetric methods. They represent all the results obtained and not selected results from a larger number of analyses.

	Extracts, Per Cent Vanillin					
	Alcoholic				Non-alcoholic	
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4	Sample No. 5	Sample No. 6
Gravimetric method . . . . .	0.26	0.26	0.14	0.11	0.80	0.29
Colorimetric method . . . . .	0.24	0.26	0.14	0.08	0.77	0.22





## PART IV

### BIOLOGICAL

## CHAPTER XLVI

### BLOOD ANALYSIS

#### NON-PROTEIN NITROGEN, UREA, CREATININE, CREATINE, URIC ACID, AND AMINO-ACID NITROGEN

IN the preceding chapters several procedures have been given for the determination of certain constituents in biological material (blood, urine, etc.). In addition to these determinations there are a number of others which should be treated in a work of the present scope. The procedures in Chapters XLVI and XLVII deal with the colorimetric analysis of the more commonly determined constituents in blood; those in Chapters XLVIII and XLIX are for urine analysis. In each case a discussion of the principles involved is given.

**Method of Drawing Blood for Analysis.**—Draw a tourniquet of soft, firm rubber tubing (or a strip of bandage) tightly around the arm of the patient about 2 ins. above the elbow, the patient keeping the fist firmly clenched. Wash the parts about the most prominent vein (usually the median basilic) with alcohol, hold the vein firmly with the thumb, and insert into the vein a sterile hypodermic needle at an angle of about 50° with the surface of the arm, keeping the opening of the needle downward or to the side. Allow the blood to run into a test tube which has its inner wall covered with a film of sodium oxalate. (See Note 4.) The blood dissolves sufficient of the oxalate to prevent clotting. Centrifuge to obtain the plasma.

#### Notes.

1. A No. 18 needle  $1\frac{1}{2}$  ins. long is suitable.
2. It is best to take the blood specimens in the morning before breakfast, to minimize the influence of food ingestion.

3. Keep all specimens in an ice box till ready for use. It is preferable to make the analysis the day a specimen is drawn, especially for sugar, since this decreases in amount on standing. However, Denis<sup>1</sup> has shown that blood may be preserved for 4 days or longer at 20 to 33° C. by adding a drop of 40 per cent formalin to each 5 cc. Denis and Bevon<sup>2</sup> recommend fluorides as a blood preservative, while Sander<sup>3</sup> and also John<sup>4</sup> recommend a mixture of sodium fluoride and thymol. Care must be taken in the selection of a preservative since they all interfere with certain methods.

4. Test tubes may be conveniently coated inside with sodium oxalate by placing in each tube  $\frac{1}{2}$  cc. of a hot saturated solution of sodium oxalate and then laying them flat on a hot-plate. The evaporating solution spatters and, upon drying, leaves a coating of oxalate on the walls of the tubes.

#### PREPARATION OF PROTEIN-FREE BLOOD FILTRATE

The total protein content of the blood is removed by precipitation with tungstic acid and filtration. The tungstic acid is formed by the interaction of sodium tungstate and sulfuric acid.

##### Reagents.

1. Sulfuric acid,  $\frac{2}{3}$  N. Dilute 35 grams of sulfuric acid, sp. gr. 1.84, to a liter, mix, and check by titration against a standard NaOH solution. (See Note 4.)

2. Sulfuric acid,  $\frac{1}{12}$  N. This solution is needed only in case the Haden modified method is to be used. It may be prepared by adding 2.5 cc. of conc. H<sub>2</sub>SO<sub>4</sub>, sp. gr. 1.84, to 1 liter of distilled water, thoroughly mixing and checking by titration against a standard NaOH solution.

3. Sodium tungstate, 10 per cent. Use Na<sub>2</sub>WO<sub>4</sub>·2H<sub>2</sub>O, taking care that it does not contain too much sodium carbonate.

**Procedure.**—Twenty milligrams of sodium or potassium oxalate per 10 cc. of blood should have been used in order to prevent coagulation. Larger amounts of oxalate or the use of citrate interferes with the deproteinization and to some extent with the uric acid determina-

<sup>1</sup> J. Biol. Chem., **44**, 203 (1920).

<sup>2</sup> J. Lab. Clin. Med., **9**, 674 (1924).

<sup>3</sup> J. Biol. Chem., **58**, 1 (1923).

<sup>4</sup> Arch. Path. Lab. Med., **1**, 227 (1926).

tion. Where uric acid is to be determined, Folin recommends that lithium oxalate be used instead of potassium oxalate.

An accurately measured quantity (5 to 15 cc.) of the oxalated blood is placed in a flask having a capacity about twenty times that of the volume of sample taken. The blood is then laked with 7 volumes of water, 1 volume of a 10 per cent solution of sodium tungstate is added and the solution mixed. From a burette or graduated pipette one volume of  $2/3$  N sulfuric acid is added, drop by drop and with constant stirring, and the flask, with a rubber stopper inserted, is shaken. If the conditions are satisfactory the shaking causes few, if any, air bubbles to form. The mixture is allowed to stand 5 minutes (10 to 20 minutes in case uric acid is to be determined). The coagulum gradually changes in color from bright red to dark brown. Failure to undergo this color change indicates incomplete coagulation and is usually due to the presence of too much oxalate. Should this occur the sample may be saved by adding 10 per cent sulfuric acid, one drop at a time, and shaking vigorously after each drop, until there is practically no foaming and the dark brown color has appeared.

The mixture is poured on a filter large enough to hold it all, care being taken to add the first few cubic centimeters to the double portion of the filter paper and allowing the whole filter to become wet before adding the remainder of the mixture. The funnel is covered with a watch-glass and the filtration continued. By this procedure the first portion of the filtrate should be as clear as water, otherwise a refiltering may be required.

Haden<sup>5</sup> has introduced an improved method for preparing protein-free filtrates. In this method fewer solutions are required, filtration is more rapid, and a larger and more nearly neutral filtrate is obtained. The modified procedure consists in mixing 7 volumes of water with the sulfuric acid, making 8 volumes of  $1\frac{1}{2}$  N  $H_2SO_4$  to be used. The 8 volumes of  $1\frac{1}{2}$  N  $H_2SO_4$  are added directly to the blood. Laking and darkening take place very rapidly. Then 1 volume of 10 per cent sodium tungstate is added and the whole thoroughly mixed and filtered as directed above.

#### Notes.

1. The graduated pipette (Fig. 54) of Folin and Wu<sup>6</sup> is very con-

<sup>5</sup> J. Biol. Chem., **56**, 469 (1923).

<sup>6</sup> J. Biol. Chem., **33**, 85 (1919).

venient for measuring the blood, the sodium tungstate solution, and the sulfuric acid.

2. If the protein-free blood filtrates are to be kept for more than 2 days, some preservative (a few drops of toluene or xylene) should be added, since the acidity of the filtrates is not high enough to prevent bacterial decomposition.

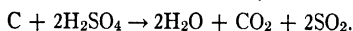
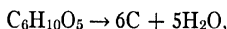
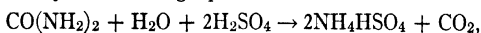
3. For optimum precipitation of protein, the *pH* of the filtrate should not be greater than 2.8. This *pH* may be conveniently determined by adding a drop of 0.04 per cent bromphenol blue to a few drops of the filtrate on a test plate. A yellow or greenish yellow color should be obtained, alkalinity being indicated by a pure blue shade.

4. The  $\frac{2}{3}$   $\text{N H}_2\text{SO}_4$  is intended to be equivalent to the sodium content of the tungstate. When equal volumes of acid and tungstate solution are mixed, practically all of the tungstic acid is set free, leaving only a little sulfuric acid. The tungstic acid is taken up almost quantitatively by the proteins and leaves the blood filtrate only slightly acid to Congo red paper.

5. In case plasma is used, only half quantities of sodium tungstate and sulfuric acid solutions should be added, the balance being made up with distilled water.

#### DETERMINATION OF NON-PROTEIN NITROGEN

This determination is made on a protein-free blood filtrate which is digested with a sulfuric acid-phosphoric acid-copper sulfate mixture. The exact course of the reactions<sup>7</sup> taking place during the digestion cannot be traced, but the acids hydrolyze the  $\text{NH}_2$ -groups to give ammonia, and also act as an oxidizing agent, converting the organic matter into carbon dioxide, water, or other volatile products. The ammonia combines at once with the acids to form ammonium acid salts. The reactions taking place may be illustrated by the following equations:



<sup>7</sup> J. H. Yoe, *Ann. chim. anal. chim. appl.* [2], **7**, 197 (1925).



FIG. 54.—Folin and Wu Pipette [*J. Biol. Chem.* **38**, 85 (1919)].

The copper sulfate gives up oxygen more readily to the organic matter than the acids do; but the acids then reoxidize the copper so that at the end of the operation the copper is still present as copper sulfate. In other words, the copper salt acts catalytically as an oxygen carrier. Koch and McMeekin use sulfuric acid and hydrogen peroxide in the digestion.<sup>8</sup>

### Reagents.

1. Sulfuric-phosphoric acid reagent. Add 300 cc. of 85 per cent phosphoric acid to 50 cc. of a 5 per cent copper sulfate solution, mix, add 100 cc. of sulfuric acid, sp. gr. 1.84, and mix. The acid must be ammonia-free. Keep the solution well stoppered and protected from ammonia in the air. Dilute with an equal volume of water before using.

2. Nessler's reagent. (a) *Preparation according to Folin and Wu.*—This reagent is an alkaline solution of the double iodide of mercury and potassium,  $\text{HgI}_2 \cdot 2\text{KI}$ . Put 150 grams of potassium iodide and 110 grams of iodine in a 500 cc. Florence flask, add 100 cc. of water and an excess of metallic mercury (140–150 grams). Shake the mixture vigorously and continuously for 7 to 15 minutes or until the dissolved iodine has about disappeared. The solution becomes quite warm. As soon as the reddish iodine solution begins to turn pale, though still red, cool under the water tap and continue shaking until the reddish iodine color has been replaced by the greenish color of the double iodide. The entire operation usually does not require more than 15 minutes. Decant the solution, wash the mercury and flask thoroughly with distilled water, dilute the solution and washings to 2 liters, and mix.

If the cooling was started in time the reagent will be sufficiently clear for diluting at once with 10 per cent alkali and water and the final solution can be used immediately for Nesslerization. From this stock solution prepare the final reagent as follows: Put 3500 cc. of 10 per cent sodium hydroxide solution in a 5-liter flask and add 750 cc. of the stock solution of the double iodide and 750 cc. of distilled water, thus making a total of 5 liters of solution. Mix the solution thoroughly.

The 10 per cent sodium hydroxide solution should be made from a saturated solution (about 55 grams  $\text{NaOH}$  per 100 cc.) which has stood

<sup>8</sup> J. Am. Chem. Soc., 46, 2066 (1924).

until the carbonate has settled, the clear solution being carefully decanted or siphoned and used. This solution should be accurate to within at least 5 per cent as indicated by checking against a standard acid.

The alkalinity of the Nessler reagent is important and should be checked against a standard acid. Twenty cubic centimeters of N HCl should require 11 to 11.5 cc. of the reagent.

(b) *Preparation according to Bock and Benedict.* Put 100 grams of mercuric iodide and 70 grams of potassium iodide in a liter flask, add about 400 cc. of water, and rotate the flask until solution is complete. Next dissolve 100 grams of sodium hydroxide in about 500 cc. of water, cool thoroughly, and add with constant shaking, to the solution in the flask, finally making up to a liter and mixing. Let the solution stand until the small amount of brownish red precipitate settles, decant or siphon off the clear supernatant liquid and use.

(c) *The modified Nessler-Folin Reagent; for Method B.*—Dissolve 22.5 grams of iodine in 20 cc. of water containing 30 grams of potassium iodide and then add 30 grams of pure mercury. Shake the mixture well, keeping it from becoming hot by holding the vessel under running tap water from time to time. As soon as the supernatant liquid has lost all of the yellow color due to iodine, decant and test a small portion by adding a few drops of the liquid to 1 cc. of a 1 per cent starch solution. A positive test for iodine shows that mercurous compounds are absent. Should a positive test not be obtained, then add iodine solution (of the same concentration as used above), a few drops at a time, until a faint excess of free iodine is present as indicated by testing a small portion with starch solution. Next dilute to 200 cc. and thoroughly mix. Add this solution to 975 cc. of an accurately prepared 10 per cent solution of sodium hydroxide, mix thoroughly, and let stand until clear.

This solution is used in the proportion of 10 cc. per 100 cc. of the solution to be Nesslerized, except in special cases where a large excess of acid is present, e.g., the direct Nesslerization methods. In these methods, the reagent should be added in an amount which will give the same alkalinity in the unknown as in the standard.

3. Sulfuric acid, 1 : 1; for method B.

4. Hydrogen peroxide, 30 per cent; for Method B. Use Merck's Superoxal or Kahlbaum's Perhydrol. Keep the solution in a cool place to avoid sudden decomposition. Do not measure out the solution

with a pipette since the vapors are very irritating to the mucous membrane, and do not allow the liquid to come in contact with the skin. The peroxide may contain an appreciable quantity of nitrogen, in which case a "blank" determination must be made and the correction applied to the result obtained with an unknown.

#### METHOD A

Place 5 cc. of the blood filtrate in a large Pyrex test tube (25 mm.  $\times$  200 mm.), preferably graduated at 35 cc. and 50 cc. The test tube should either be dry or rinsed with alcohol so as to reduce the danger of bumping. Drop a quartz pebble into the test tube, add 1 cc. of the diluted sulfuric-phosphoric acid reagent, or  $\frac{1}{2}$  cc. of the undiluted, and boil vigorously over a micro-burner till dense white fumes begin to fill the tube. Three to 7 minutes will be required. As soon as the fumes almost fill the tube, lower the flame till the solution just boils, cover the tube with a small watch-glass, and continue gentle heating for 2 minutes, counting from the moment the tube became filled with fumes. Should the oxidation be incomplete at the end of 2 minutes, continue gentle heating until the solution is almost colorless. Usually 20 to 40 seconds are required for the solution to become colorless. Remove the flame at the end of 2 minutes, allow the solution to cool for 70 to 90 seconds, add 15 to 25 cc. of water, cool to about room temperature, and dilute to the 35 cc. mark with water. (See Note 1.) Now add 15 cc. of Nessler's reagent, stopper with a clean rubber stopper, and mix. In case the solution is turbid, centrifuge before Nesslerizing and matching it against the standard.

A standard containing 0.3 mg. of nitrogen is generally employed. Transfer to a 100 cc. graduated flask 3 cc. of a standard ammonium sulfate solution made by dissolving 0.4716 gram of specially purified ammonium sulfate in 1 liter of ammonia-free distilled water. (See Note 2.) This solution contains 0.1 mg. of N per cubic centimeter. Next add 2 cc. of the diluted sulfuric-phosphoric acid reagent, dilute to about 60 cc., add 30 cc. of Nessler's reagent, dilute to the mark, and thoroughly mix. The standard and sample solutions should be Nesslerized simultaneously.

*Calculation.*—The unknown or sample solution may be set at 20 mm. Then the colorimeter reading multiplied by 0.3 and divided by 20 gives the non-protein nitrogen in 1 cc. of blood, since 0.5 cc. (the



amount of blood represented in 5 cc. of the blood filtrate), Nesslerized at a volume of 50 cc., is equivalent to 1 cc. Nesslerized at a volume of 100 cc. Dividing the reading by 20 and multiplying by 30 (0.3 times 100) gives the non-protein nitrogen per 100 cc. of blood.

To eliminate calculation, set the *unknown* at 30 mm. The colorimeter reading of the standard equals the number of milligrams of non-protein N per 100 cc. of blood.

#### METHOD B: PROCEDURE OF KOCH AND McMEEKIN<sup>9</sup>

Place 5 cc. of the blood filtrate in a large Pyrex test tube (25 mm.  $\times$  200 mm.), add 1 cc. of sulfuric acid (1 : 1), evaporate off the water on a sand-bath or by heating gently over a free flame with shaking. Finally heat over a micro-burner till dense white fumes are given off, add 1 to 3 drops of 30 per cent hydrogen peroxide and again heat to boiling. Should the solution discolor, repeat the addition of hydrogen peroxide and then boil gently for 5 minutes. Cool the solution, transfer it quantitatively to a 50 cc. volumetric flask with about 35 cc. of water, and then add with shaking 12 cc. of the modified Nessler-Folin reagent. Dilute to the mark and thoroughly mix. A test tube graduated at 35 cc. and 50 cc. may be used as in Method A. Prepare a standard solution containing 0.1 to 0.3 mg. of N by adding 1 cc. of 1 : 1 sulfuric acid to a measured quantity of a standard ammonium sulfate solution diluted to 35 cc. and Nesslerized as directed above. After unknown and standard have stood 5 to 20 minutes, they are compared in a colorimeter. The calculation is the same as in Method A. If the hydrogen peroxide contains nitrogen, a correction for it must be made.

#### Notes.

1. McCrackan, Passamaneck and Harman<sup>10</sup> recommend distillation with sodium hydroxide preliminary to Nesslerization, and describe an apparatus for the process. (Fig. 68, p. 501.)

2. Care must be taken to obtain a pure ammonium salt for the standard solution, since all ammonium salts contain pyridine bases.

<sup>9</sup> J. Am. Chem. Soc., **46**, 2066 (1924). Rose uses a stronger acid digestion mixture, containing perchloric acid [J. Biol. Chem., **64**, 253 (1925)]. See also Mears and Hussey, J. Ind. Eng. Chem., **13**, 1054 (1921); and Yoe, Ann. chim. anal. chim. appl. [2], **7**, 193 (1925).

<sup>10</sup> J. Lab. Clin. Med., **11**, 678 (1926).

The latter titrate like ammonia but they do not react with Nessler's reagent. To prepare *pure* ammonium sulfate, decompose an ammonium salt of a high grade with sodium hydroxide and absorb the liberated ammonia gas in pure sulfuric acid. Add alcohol to precipitate the salt, filter, suck "dry," dissolve it in water, reprecipitate with alcohol, and again filter. Dry the salt in a desiccator over concentrated sulfuric acid. Pyridine-free ammonium salts can now be obtained on the market.<sup>11</sup>

#### DETERMINATION OF UREA

The urea is converted into ammonium carbonate by means of the enzyme urease, in the presence of a phosphate buffer solution which also catalyzes the conversion. The ammonia may be distilled off, removed by aëration, or determined directly by Nesslerization. The autoclave method is also given.

##### METHOD A: DISTILLATION PROCESS OF FOLIN AND WU<sup>12</sup>

###### Reagents.

1. Hydrochloric acid, 0.05 N (and 0.5 N for Method C).
2. *Buffer Solution A.* Dissolve 69 grams of monosodium phosphate and 179 grams of crystallized disodium phosphate in about 800 cc. of warm distilled water, dilute to a liter and mix. *Buffer Solution B.*—Dissolve 14 grams of sodium pyro-phosphate,  $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ , in enough 0.5 N phosphoric acid to make 100 cc. Make the 0.5 N acid by diluting 20 cc. of 85 per cent phosphoric acid to a liter and titrating 5 cc. with standard alkali solution. Upon the basis of this titration dilute the acid to 0.5 N.
3. Urease solution. Place about 3 grams of "Permutit" in a flask, wash it once with 2 per cent acetic acid and then twice with water. Add 5 grams of finely divided jack bean meal, 100 cc. of 15 per cent alcohol, shake gently and continuously for about 15 minutes, pour on a large filter, and cover with a watch-glass. This solution may be kept about a week at room temperature or from 4 to 6 weeks in an ice box.
4. Borax. Use a saturated solution.

<sup>11</sup> Cf. Hawk and Bergeim, *Practical Physiological Chemistry*, 9th ed., p. 715. P. Blakiston's Son and Co., Philadelphia, 1926.

<sup>12</sup> J. Biol. Chem., **38**, 81 (1919).

5. Paraffin oil.
6. Sodium carbonate, 10 per cent; for Method B.
7. Sodium hydroxide, 10 per cent; for Method C.

**Procedure.**—Place 5 cc. of the tungstic acid blood filtrate in a large Pyrex test tube which has been thoroughly rinsed with nitric acid and then with water. Add 2 drops of the Buffer Solution A, 1 cc. of

the urease solution, immerse the tube in warm water (40–55° C.) and leave it there for 5 minutes, or allow it to stand for 15 minutes at room temperature.

A convenient way to obtain the ammonia formed from the urea is to distill, without a condenser, using a test tube graduated at 25 cc. and containing 2 cc. of 0.05 N hydrochloric acid as a receiver. Figure 55 shows a compact and convenient arrangement for this distillation. A groove may be cut in the side of the stopper of the receiving tube to allow steam to escape. Watson and White<sup>13</sup> have used a modification of the Folin and Wu apparatus.

Put a small dry pebble in the urease blood filtrate, add 1 or 2 drops of paraffin oil and 2 cc. of saturated borax solution, insert firmly the rubber stopper carrying the delivery tube and receiver, and then boil the mixture fairly rapidly and at a uniform rate for 4 minutes. Never

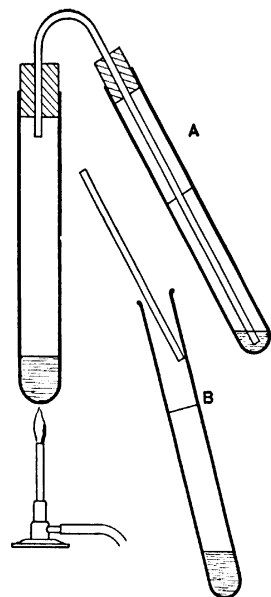


FIG. 55.—Folin and Wu Distillation Apparatus [J. Biol. Chem., **38**, 96 (1919)]. A, at beginning; B, toward end of distillation.

turn down the flame during a distillation, and regulate the rate of boiling so that the emission of steam from the receiver never begins before the end of 3 minutes. At the end of 4 minutes slip the receiver tube from the stopper and let it rest in a standing position while the distillation is continued for 1 minute. Rinse the lower end of the delivery tube, cool the distillate with running water, and dilute to about 20 cc. Place 0.3 mg. of N (3 cc. of standard ammonium sulfate

<sup>13</sup> J. Biol. Chem., **45**, 465 (1921).

solution—see p. 449) in a 100 cc. volumetric flask and dilute to about 75 cc. Add 2.5 cc. of Nessler's reagent to the sample solution and 10 cc. to the standard. Dilute both solutions to the mark, thoroughly mix, and compare them in a colorimeter.

*Calculation.*—If the unknown is set at 20 mm., then the colorimeter reading divided by 20 is multiplied by 15. This gives the urea nitrogen in milligrams per 100 cc. of blood. It should be noted that in the above procedure the sample representing 0.5 cc. of blood is Nesslerized at 25 cc., whereas in the case of non-protein nitrogen it is Nesslerized at 50 cc. Therefore, the same colorimeter reading represents only half as much nitrogen in the urea determination as in the non-protein nitrogen determination.

By setting the unknown at 15, the colorimeter reading of the standard gives directly the milligrams of urea N per 100 cc. of blood.

#### METHOD B: AUTOCLAVE PROCESS

This method is convenient when a large number of determinations are to be made or when creatine determinations are also to be made.

Transfer 5 cc. of the blood filtrate to a large Pyrex test tube, add 1 cc. of 1 N hydrochloric acid, cover with tin foil and heat in an autoclave at 150° C. for 10 minutes. Distill off the ammonia as directed above, except that 2 cc. of 10 per cent sodium carbonate must be used instead of the borax solution, on account of the hydrochloric acid added.

#### METHOD C: AERATION PROCESS

Instead of distilling the ammonia formed by the action of blood urea and urease or by heating under pressure, it may be driven into the receiver by passing a current of air through the solution after the addition of an alkali. This process gives perfectly reliable results, provided a good current of air is used.

Add a little paraffin oil and 1 or 2 cc. of 10 per cent sodium hydroxide to the decomposed blood filtrate contained in a large test tube. Connect with a smaller test tube (marked at 25 cc.) containing 2 cc. of 0.5 N hydrochloric acid. The connection is made as illustrated in Fig. 72 on page 512. Pass the air through slowly for 1 minute and then for 10 to 15 minutes almost as rapidly as the apparatus will permit. Rinse the connecting tube, dilute the contents of the receiver to

about 20 cc., add 2.5 cc. of Nessler's reagent, dilute to the 25 cc. mark, mix, and compare the color against a standard in the usual manner.

#### METHOD D: KARR'S<sup>14</sup> DIRECT NESSLERIZATION OF THE FOLIN-WU FILTRATE

To 5 cc. of the Folin-Wu blood filtrate in a test tube add 1 drop of Buffer Solution B and either a strip of urease paper (see Note 3) or 5 drops of urease solution (prepared as directed in Note 4).

Treat similarly in another tube 5 cc. of a standard urea solution (see Note 5), prepared by diluting 5 cc. of a stock solution (containing 0.1286 gram urea in 200 cc.) to 100 cc. with water (5 cc. = 0.075 mg. N). Place the tubes in a water-bath at 50° C. for 15 minutes. Then transfer the contents of the tubes, with rinsing, to test tubes (or ordinary 25 cc. graduated cylinders) graduated at 22.5 and 25 cc. Dilute each tube to the 22.5 cc. mark and add Nessler's reagent to the 25 cc. mark. Mix and in about 1 minute make the colorimeter reading.

*Calculation.*—By setting the unknown at 15 mm., the reading of the standard gives directly the urea N per 100 cc. of blood. In case less than 5 cc. of filtrate (0.5 cc. blood) was used, make the necessary correction.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.075 \times \frac{100}{0.5} = \text{mg. urea N per 100 cc. of blood.}$$

#### Notes.

1. Blood filtrates high in urea nitrogen (35 mg., or over, per 100 cc.) give a turbid solution upon Nesslerization. In such cases, repeat the determination, using a smaller amount of filtrate.

2. If a large number of determinations are made daily, it is advisable to use an artificial permanent standard (see original paper) which is checked daily against a standard urea solution.

3. Urease paper may be prepared according to Folin's directions as follows: Shake 15 grams of jack bean meal with 5 grams of "Permutit" in 100 cc. of 15 per cent alcohol for 15 minutes on a mechanical shaker or a half hour by hand. Filter through a fluted filter paper (best in an ice box overnight), pour the filtrate in an open dish and draw pieces

<sup>14</sup> Karr, J. Lab. Clin. Med., 9, 3 (1924). Roe and Irish, *ibid.*, 11, 1087 (1926), remove traces of protein by adsorption on tricalcium phosphate and thus minimize the turbidity.

of heavy filter paper (ammonia-free) through it. Hang the pieces of paper up to dry and when dry cut them into strips about 25 mm. by 40 mm.

4. The urease solution used in Method D is prepared as directed for urease paper in the preceding note. The solution is not as stable as the urease paper. It should be made up fresh about every 2 weeks and should be kept on ice. Enzyme paper is preferable if determinations are made only occasionally.

5. Since mercury has a "poisoning" action on urease, separate tubes must be used for Nesslerization. In case of doubt, the tubes should be cleaned with strong nitric acid before use.

#### METHOD E: MYERS' DIRECT AERATION METHOD<sup>15</sup>

##### Reagents.

1. Hydrochloric acid, 10 per cent.
2. Sodium carbonate. Use a saturated solution.
3. Caprylic alcohol.
4. Urease reagent. Solid urease may be obtained from soy bean meal according to the method of Van Slyke and Cullen,<sup>16</sup> or may be purchased in powder or tablet form from Hynson, Westcott, and Dunning, Baltimore, Md., and the Arlington Chemical Co., Yonkers, N. Y. To prepare the urease reagent, dissolve 2 grams of the enzyme preparation, 0.6 gram of dipotassium hydrogen phosphate, and 0.4 gram of monopotassium dihydrogen phosphate in 10 cc. of water. Cover the slightly opalescent solution with toluene. The reagent may be kept 2 weeks without losing its activity.
5. Standard ammonium sulfate solution. Dissolve 0.944 gram of ammonium sulfate (pyridine-free) in ammonia-free water, dilute to a liter, and thoroughly mix. Five cubic centimeters of this solution contain 1 mg. of N. See Note 2, p. 450.

**Procedure.**—The set-up of the apparatus is as illustrated in Fig. 56. Add 1 cc. of the urease reagent to a lipless test tube which just fits into a 100 cc. ungraduated cylinder. Then add 2 cc. of the oxalated blood and heat the tube for 15 minutes at 50° C.

<sup>15</sup> V. C. Myers, *Practical Chemical Analysis of the Blood*, 2d ed., C. V. Mosby Co., St. Louis, 1924. This method is based on those of Folin and Denis, *J. Biol. Chem.*, **11**, 527 (1912); Marshall, *ibid.*, **15**, 487 (1913); and Van Slyke and Cullen, *ibid.*, **19**, 211 (1914).

<sup>16</sup> *J. Biol. Chem.*, **19**, 211 (1914).

Add 15 cc. of distilled water and 2 or 3 drops of 10 per cent hydrochloric acid to a 100 cc. lipless graduated cylinder, stopper with a two-holed stopper carrying a glass tube which extends almost to the bottom of the cylinder. Place the test tube containing the digestion mixture in the ungraduated cylinder and connect the two cylinders. Add 5 drops of caprylic alcohol and 4 or 5 cc. of saturated sodium carbonate solution to the tube containing the blood digest, and stopper quickly. The cylinder containing the digested mixture is connected with a wash bottle two-thirds full of dilute sulfuric acid. The latter will remove ammonia from the incoming air. Connect the outlet tube of the graduated cylinder to a suction pump and draw air slowly through the apparatus, increasing the speed so that at the end of about 2 minutes

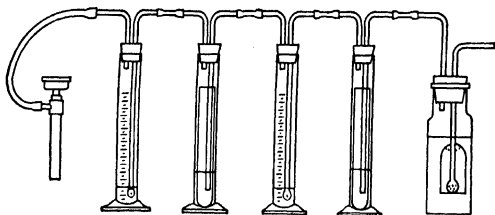


FIG. 56.—Myers' Aeration Apparatus (Myers, *Practical Chemical Analysis of the Blood*, 2d., p. 44. C. V. Mosby Co., St. Louis, 1924).

the maximum rate permissible has been reached. Continue the aëration for 30 minutes and then disconnect the apparatus.

To a 100 cc. volumetric flask add 5 cc. of the standard ammonium sulfate solution, 50 to 60 cc. of ammonia-free water, 20 cc. of Nessler's solution (see p. 447), dilute to the mark with ammonia-free water and mix thoroughly. At the same time add 7 or 8 cc. of Nessler's solution to the unknown, dilute to the 25 cc. mark, and mix thoroughly. Compare the two solutions in a colorimeter.

- In case the urea nitrogen is high, use more of Nessler's solution and dilute till the color approximates that of the standard.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 1 \times \frac{100}{2} \times \frac{25}{100} = \text{mg. urea N per 100 cc. of blood.}$$

If the unknown is set at 12.5 mm., the reading of the standard gives directly the number of milligrams of urea nitrogen per 100 cc. of blood.

Note that for high concentrations the dilution factor  $\left(\frac{25}{100}\right)$  must be corrected accordingly and the whole equation employed in the calculation.

#### DETERMINATION OF PRE-FORMED CREATININE

The method is based upon the deep red color produced when creatinine solutions are treated with picric acid in alkaline solution. This reaction<sup>17</sup> is known as the Jaffé reaction, and is due to the formation of a red tautomer of creatinine picrate.<sup>18</sup> The production of this tautomer "is dependent upon the formation of a salt, a keto-enol change within the creatinine molecule, and a change in the picric acid molecule involving the hydrogens in the *meta* positions and, probably, all three nitro-groups."<sup>19</sup>

#### Reagents.

1. Picric acid. Use a saturated solution made with purified picric acid. Distilled water may be allowed to stand in contact with picric acid, or a 1.2 per cent solution may be made.

Picric acid may be purified by one of the following methods:

(a) *Method of Halverson and Bergeim.*<sup>20</sup>—Add 700 cc. of distilled water to 50 grams of picric acid and boil until a clear solution is obtained. While the solution is boiling, add 10 cc. of concentrated hydrochloric acid. Cool, wash the crystals by decantation with 100 cc. of distilled water, and repeat the crystallization. Finally transfer the crystals to a Büchner funnel, wash them with about 150 cc. of water, and dry in a desiccator or between filter papers. •

(b) *Method of Folin and Doisy.*—Place in a beaker of at least 4 liters capacity about a pound of dry picric acid (or about 600 grams of the wet acid), add boiling water until the beaker is nearly full and then add 200 cc. of saturated (50 per cent) sodium hydroxide solution.

<sup>17</sup> M. Jaffé, Z. physiol. Chem., **10**, 391 (1886).

<sup>18</sup> I. Greenwald and J. Gross, J. Biol. Chem., **59**, 601 (1924).

<sup>19</sup> I. Greenwald, J. Am. Chem. Soc., **47**, 1448 (1925).

<sup>20</sup> P. B. Hawk, and O. Bergeim, Practical Physiological Chemistry, 9th ed., p. 890. P. Blakiston's Son and Co., Philadelphia, 1926.



Thoroughly stir the mixture, and, if necessary, heat it until the picric acid completely dissolves, giving a deep red solution of sodium picrate. Add to the hot solution 200 grams of sodium chloride, slowly and with stirring. Place the vessel in cold running water and cool the solution to about 30° C. Filter on a large Büchner funnel, wash several times with small volumes of 5 per cent sodium chloride solution, transfer the picrate to a large beaker, add boiling water until the beaker is almost full and stir till solution is complete. Add, with stirring, 50 cc. of 10 per cent sodium hydroxide solution, and then 100 grams of sodium chloride. Cool, filter, and wash with sodium chloride solution as before. Once more repeat the solution and precipitation of the sodium picrate, but wash this time with distilled water instead of sodium chloride solution.

The purified picrate is then dissolved in several liters of boiling distilled water and the hot solution filtered on a large folded filter, the filtrate being collected in a large flask. Dilute 100 cc. of concentrated sulfuric acid with 200 cc. of water and add the diluted acid to the hot filtrate. Picric acid begins to precipitate at once. Cover the mouth of the flask with an inverted beaker and cool the mixture under the water tap to about 30° C. Filter with suction as before and wash with small volumes of distilled water till free from sulfuric acid. Dry the crystals in a desiccator or between filter papers.

(c) *Benedict's Method*.—Place about 400 grams of moist, "technical" grade picric acid in a 2-liter Pyrex flask and add 1 liter of pure benzene. Heat on an electric hot-plate, with shaking now and then, until the acid dissolves, leaving a residue of impurities and water which settles quickly to the bottom when the flask is removed from the hot-plate. Decant the hot solution through a large fluted filter which has been "moistened" with benzene, care being taken to transfer as little of the sediment as possible on to the filter. A hot-water funnel is convenient for this filtration but is not necessary. If used, be sure to turn out the flame just prior to filtering, since the benzene picric acid solution is highly inflammable.

The filtrate should be received in a 2-liter beaker. Cover with a large watch-glass and heat on an electric hot-plate to redissolve the picric acid which has begun to crystallize. Remove the beaker from the hot-plate and let stand for several hours (better overnight). The picric acid crystallizes on the bottom and sides of the beaker and may be drained free of the excess of benzene without need of filtering.

Wash the crystals twice by decantation, using about 75 cc. of benzene for each washing, and finally drain them for about 30 minutes. Remove the last trace of benzene by drying in an air-bath at about 80° for several hours. Powder the crystals by *gentle* rubbing in a mortar and keep the product in a dark-brown glass stoppered-bottle. To recover the benzene, distill (preferably *in vacuo*) from a water-bath.

2. Sodium hydroxide, 10 per cent.

3. Standard creatinine solution. Dissolve 0.100 gram of pure creatinine in 100 cc. of 0.1 N hydrochloric acid and mix thoroughly. Transfer 3 cc. of this solution to a 500 cc. volumetric flask, add 50 cc. 0.1 N hydrochloric acid, dilute to the mark with water, and mix thoroughly. Pour the solution into a bottle, add 4 or 5 drops of toluene or xylene and stopper. Five cubic centimeters of this solution (0.03 mg. of creatinine) are diluted with 15 cc. of water and mixed thoroughly. This gives the standard usually needed for human blood, for it covers the range of 1 to 2 mg. per 100 cc. In the case of bloods with high creatinine content, it is best to use smaller volumes of blood filtrate and dilute with water to the usual 10 cc., taking this into consideration in making the calculation below.

4. Standard creatinine zinc chloride. A better standard can be prepared from creatinine zinc chloride than from creatinine. Pure creatinine zinc chloride may be prepared as follows according to the method of Edgar:<sup>21</sup> Commercial creatine is ground in a mortar with an equal weight of anhydrous zinc chloride. The mixture is then placed in a beaker, dish, or casserole, and is heated over a small flame or sand-bath, with constant stirring. As the temperature is raised the mixture begins to melt, and around 120–130° C. (depending somewhat on the rate of heating and the moisture which may have been absorbed) becomes a viscous mass from which bubbles of water vapor are given off. Within a few minutes the mass suddenly solidifies to a perfectly dry residue consisting of creatinine zinc chloride (containing, of course, the excess zinc chloride). The whole reaction is complete within about 5 minutes after beginning to raise the temperature. If crude creatinine zinc chloride is desired, it is only necessary to leach the mass with a little cold water or aqueous alcohol to dissolve out the excess zinc chloride. If a pure product is desired the residue may be dissolved directly (following Folin) in about ten times its weight of boiling 25 per cent acetic acid, and 2 volumes of alcohol are added to

<sup>21</sup>J. Biol. Chem., **56**, 1 (1923).

the mixture. On cooling, crystalline creatinine zinc chloride separates in practically quantitative yield (based on the original creatine), and of a purity comparable with the best product obtained by repeated crystallization.

To prepare a standard solution, dissolve 1.602 grams of the pure creatinine zinc chloride in 0.1 N HCl, dilute to a liter with 0.1 N HCl and mix thoroughly. One cubic centimeter of this solution contains 1 mg. of creatinine.

Aqueous solutions of creatinine zinc chloride, like pure creatinine solutions, slowly change in concentration owing to partial creatine formation, but this can be readily overcome by adding acid to the solution or by using 0.1 N HCl instead of water.

**Procedure.**—Make up a fresh alkaline picrate solution by adding 5 cc. of 10 per cent sodium hydroxide to 25 cc. of a saturated solution of pure picric acid and mixing. Place 10 cc. of the blood filtrate in a small flask. In another flask place 5 cc. of the standard creatinine solution and dilute to 20 cc. Then add 5 cc. of the freshly prepared alkaline picrate solution to the blood filtrate, and 10 cc. to the diluted creatinine solution. After standing 8 to 10 minutes, compare the solutions in a colorimeter. Be sure that the two fields of the colorimeter are equal when both cups contain the standard creatinine picrate solution and are at the same height. The color matching should be completed within 15 minutes after adding the alkaline picrate solution.

*Calculation.*

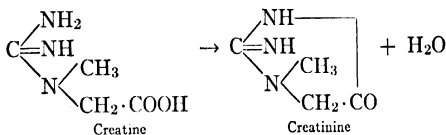
$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 1.5 = \text{mg. creatinine in 100 cc. of blood.}$$

It should be noted that the standard is diluted to twice the volume of the unknown, so that each 5 cc. of the standard (containing 0.03 mg. of creatinine) corresponds to 0.015 mg. in the blood filtrate.

By setting the unknown at 30 mm., the reading of the standard divided by 20 or multiplied by 0.05 gives the number of milligrams of creatinine in 100 cc. of blood.

## DETERMINATION OF CREATINE

When creatine is heated with acid it is converted into creatinine by dehydration.



By determining the creatinine content before and after the acid treatment, the amount of creatine originally present can be calculated.

## Reagents.

1. Hydrochloric acid, 1 N.
2. Alkaline picrate. Prepare as needed according to directions given in the preceding determination.
3. Standard creatinine solution. Prepare as directed in the preceding determination.

**Procedure.**—To 5 cc. of blood filtrate in a test tube graduated at 25 cc., add 1 cc. of 1 N hydrochloric acid, cover with tin foil and heat in an autoclave at 155° C. for 10 minutes, or at 130° C. for 20 minutes. Cool, add 5 cc. of alkaline picrate solution, let stand 8 to 10 minutes, dilute to 25 cc., and mix. To prepare a standard comparison solution, add 2 cc. of 1 N hydrochloric acid and 10 cc. of alkaline picrate solution to 10 cc. of standard creatinine solution contained in a 50 cc. volumetric flask, let stand 10 minutes, dilute to 50 cc., and mix thoroughly. The standard solution must be ready for use as soon as the unknown is ready.

## Calculation.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 6 = \left\{ \begin{array}{l} \text{"Total creatinine" in mg. per 100 cc.} \\ \text{of blood.} \end{array} \right.$$

"Total creatinine" — Preformed creatinine = creatine in terms of creatinine.

One gram of creatinine  $\approx$  1.159 grams of creatine.

One gram of creatine  $\approx$  0.8626 gram of creatinine.

## DETERMINATION OF URIC ACID BY DIRECT COLORIMETRIC METHODS

The methods are based upon the blue color obtained when uric acid solutions are treated with an arseno-phosphotungstic acid reagent and sodium cyanide.<sup>22</sup> The reaction is more specific for uric acid than either arsenic, tungstic, or phosphoric-tungstic acid alone and the intensity of the color is much greater (nearly seven times) than that obtained with the old phosphoric acid reagent. Moreover, the new reagent is scarcely affected by a typical polyphenol such as resorcinol in the presence of uric acid.

## METHOD A (BENEDICT)

## Reagents.

1. Sodium cyanide, 5 per cent. (Poison!) Dissolve 50 grams of sodium cyanide in water containing 2 cc. of ammonia, sp. gr. 0.90, and dilute to a liter. Make up a fresh solution about every 2 months.

2. Uric acid reagent. Dissolve 100 grams of pure sodium tungstate in about 600 cc. of water, add 50 grams of pure arsenic pentoxide, 25 cc. of 85 per cent phosphoric acid, 20 cc. of concentrated hydrochloric acid, boil the mixture for about 20 minutes, cool, dilute to a liter, and mix. The reagent apparently keeps indefinitely.

3. Standard uric acid solutions. Prepare a stock solution as follows: Nine grams of pure crystallized disodium hydrogen phosphate and 1 gram of pure crystallized sodium dihydrogen phosphate are dissolved in about 250 cc. of hot water. Filter the solution if it is not clear. Dilute with hot water and pour the clear hot solution into a liter volumetric flask containing 200 mg. of pure uric acid suspended in a few cubic centimeters of water. Shake for a few minutes until the acid dissolves, cool, add *exactly* 1.4 cc. of glacial acetic acid, dilute to the mark, and mix thoroughly. This solution contains 0.2 mg. of uric acid per cubic centimeter. Four or 5 cc. of chloroform are added to prevent the growth of bacteria or molds. Unless kept in an excessively warm room, the solution should keep about 2 months.

*Preparation of a Dilute Standard.*—Transfer 10 cc. of the above stock solution to a 500 cc. volumetric flask containing 200 to 300 cc.

<sup>22</sup> S. R. Benedict, J. Biol. Chem., **51**, 187 (1922); **54**, 233 (1922); **64**, 215 (1925); S. R. Benedict and E. Franke, *ibid.*, **52**, 387 (1922); O. Folin, *ibid.*, **54**, 153 (1922); O. Folin, Laboratory Manual of Biological Chemistry, 4th ed., p. 247. D. Appleton and Co., New York, 1926.

of distilled water, add 25 cc. of dilute HCl (1 : 10), dilute to the mark, and thoroughly mix. This solution contains 0.02 mg. of uric acid in 5 cc. Prepare the solution fresh every 2 weeks.

**Procedure.**—Transfer to a test tube (18 to 20 mm. diam.) 5 cc. of blood filtrate and add 5 cc. of water. Five cubic centimeters of standard uric acid solution (0.02 mg. uric acid per 5 cc.) are likewise diluted to 10 cc. Add to both unknown and standard 4 cc. of the ammoniacal 5 per cent sodium cyanide solution (poison!) and then 1 cc. of the uric acid reagent. Mix at once the contents of each tube by one inversion and place them in boiling water. (See Note.) After heating for 3 minutes, place the tubes in a large beaker of cold water for 3 minutes and then compare them in a colorimeter (preferably within 5 minutes after removing from the cold water).

In case the solutions show a clouding (usually caused by too much oxalate), repeat the test, adding another 5 cc. of water to both the standard and unknown just before heating them.

**Calculation.**—Using a standard containing 0.02 mg. of uric acid and 5 cc. of 1 : 10 blood filtrate, the calculation is made as follows:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 4 = \text{mg. uric acid in 100 cc. of blood.}$$

If the unknown is set at 20 mm., then multiplying the reading of the standard by 0.2 gives the final result.

**Note.**—Brown<sup>23</sup> allows the solution to stand for 20 minutes at room temperature instead of heating. Twice the amount of filtrate is used. He obtains lower values than those by the above procedure.

#### METHOD B (FOLIN)

##### Reagents.

1. Sodium cyanide, 15 per cent. Dissolve 150 grams of a good grade of sodium cyanide (poison!) in 700 to 800 cc. of 0.1 N NaOH solution and dilute to a liter with 0.1 N NaOH. Let stand 1 or 2 days before using. Prepare fresh once a month.

2. Uric acid reagent. The reagent described on page 462 is satisfactory for ordinary clinical analysis. For the highest accuracy pre-

<sup>23</sup> J. Biol. Chem., 68, 123 (1926).

pare the reagent as follows: Put 100 grams of sodium tungstate,  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ , and 160 cc. of water in a 500 cc. flask. Add 50 cc. of 85 per cent phosphoric acid, little by little, and with cooling under the water tap. Then connect the flask with a gas wash bottle half filled with water and attached to a  $\text{H}_2\text{S}$  generator. The flask should be stoppered with a two-hole rubber stopper carrying an inlet tube extending to the bottom of the flask and an outlet tube which can be opened or closed with a pinchcock. Carefully displace the air in the flask with  $\text{H}_2\text{S}$ . Then close pinchcock and bubble  $\text{H}_2\text{S}$  slowly into the solution. Leave the set-up overnight. The solution takes on a deep blue color. The next morning disconnect the apparatus and filter the solution into a 500 cc. flask. Let the precipitate drain but do not wash it. Insert a 10 cm. funnel and place in it a 200 cc. flask filled with cold water. This serves as a condenser. Boil the solution gently over a micro-burner for 1 hour and filter the hot solution to remove the small quantity of molybdenum sulfide which separates out during boiling. Wash the precipitate with small volumes of water until most of the blue color has been removed from the filter paper. Heat the filtrate to boiling, remove the flame and decolorize by adding bromine (only 2 to 3 drops at a time) and shaking vigorously. As soon as an excess of bromine has been added the solution takes on a clear light yellow color. Remove the excess of bromine by boiling gently for 10 minutes; then allow the solution to cool.

Place 25 grams of lithium carbonate in a liter beaker, add 50 cc. of 85 per cent phosphoric acid, and 200 cc. of water, boil off the carbon dioxide, and cool. Mix this solution with the one above and dilute to a liter. This reagent should give only a very faint bluish tint with 0.2 mg. of resorcinol.

When uric acid in silver precipitates from human urine is to be determined, or when clinical determinations on human blood are to be made, the removal of molybdenum may not be necessary, since uric acid gives a much stronger color than phenols. The presence of a large trace of molybdenum may cause errors up to 1 mg. per 100 cc. of blood.

3. Stock solution of uric acid. Accurately weigh on a watch-glass 1 gram of uric acid and transfer it to a small dry funnel inserted in a liter volumetric flask and shake the uric acid into the flask as well as possible. Dissolve 0.6 gram of lithium carbonate in about 120 cc. of hot water, filter, dilute with about 60 cc. of water, and heat to  $65^\circ \text{C}$ .

Warm the liter flask in hot water and add the hot lithium carbonate solution, rinsing the watch-glass and funnel free of the last trace of uric acid. Shake the flask until the uric acid has dissolved, cool, dilute to about 800 cc., add 10 cc. of clear 37 to 40 per cent formaldehyde (Merck) and mix. Dilute 15 cc. of sulfuric acid, sp. gr. 1.84, with about 100 cc. of water, cool, add to the urate-formalin solution, dilute to a liter, and mix thoroughly. This solution will keep for at least several months.

4. Standard uric acid solution (5 cc. = 0.02 mg.). Transfer by means of an accurate Ostwald pipette 1 cc. of the above stock solution to a 250 cc. volumetric flask containing about 150 cc. of distilled water. Add 10 cc. of  $\frac{2}{3}$  N  $\text{H}_2\text{SO}_4$ , dilute to 250 cc., and mix thoroughly. This solution will keep at least a week. Five cubic centimeters of it contain 0.02 mg. of uric acid.

**Procedure.**—Add 5 cc. of blood filtrate to a test tube graduated at 25 cc., and 5 cc. of the standard uric acid (0.02 mg. uric acid) to a similar tube. Add from a burette 2 cc. of the alkaline sodium cyanide solution to each tube and 2 cc. of water. Mix well, and add 1 cc. of uric acid reagent to each tube, dropping it from a burette directly into the solution (not down the sides of the tubes). Let the solutions stand for 2 minutes at room temperature and then put them in a boiling water-bath (a large beaker about two-thirds full of boiling water) for 2 minutes, but not longer. Cool at once, dilute to the 25 cc. mark, mix, and compare in a colorimeter.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 4 = \text{mg. uric acid per 100 cc. of blood.}$$

If the unknown is set at 20 mm., then multiplying the reading of the standard by 0.2 gives the result. Should the standard reading be greater than 40, repeat the determination, using 3 cc. of filtrate and 2 cc. of water. If the reading is less than 10, use 10 cc. of filtrate and 5 cc. of standard with 5 cc. of water.

**Notes.**

1. Direct determinations on blood filtrates have been criticized as giving too high values. This is said to be due to the presence of



thiasine (a sulfur-containing substance) which gives a color reaction similar to the one produced by uric acid.<sup>24</sup>

2. The removal of traces of molybdenum<sup>25</sup> from the uric acid reagent increases its specificity.

#### METHOD C (FOLIN'S ISOLATION METHOD)<sup>26</sup>

Since certain other substances present in blood filtrate and urine may produce a blue color with phosphotungstic reagent similar to that produced by uric acid, it is sometimes desirable to separate the uric acid from the interfering substances. This is accomplished by precipitating the uric acid as silver urate. The silver urate is then treated with sodium chloride in hydrochloric acid solution. The uric acid thus set free is determined colorimetrically. This procedure gives more accurate results than the preceding ones.

##### Reagents.

1. Sodium chloride, 10 per cent in 0.1 N HCl.
2. Silver lactate. Dissolve 100 grams of silver lactate in 700 cc. of warm water. Add 100 cc. of 10 per cent sodium hydroxide to 100 cc. of 85 per cent lactic acid and pour the resulting solution into the silver lactate solution. Dilute to a liter and allow to stand till the sediment settles. Decant and use the clear solution.

For other reagents see Method B (Folin).

**Procedure.**—Place 5 cc. of the blood filtrate in a centrifuge tube, add 7 cc. of the silver lactate solution (without stirring), let settle for 1 to 2 minutes and centrifuge. The precipitate contains all of the uric acid. Decant the clear liquid as completely as possible, add 1 cc. of the NaCl-HCl solution, stir *thoroughly* with a small glass rod; add 4 cc. of water, again stir, and then centrifuge,

Pour off the supernatant solution as completely as possible into a test tube graduated at 25 cc. Measure 5 cc. of the standard uric acid solution into a similar test tube and complete the procedure as directed in Method B.

**Calculation.**—Same as in Method B.

<sup>24</sup> Bulmer, Eagles, and Hunter, *J. Biol. Chem.*, **63**, 17 (1925); Benedict, *ibid.*, **64**, 215 (1925); Benedict, Newton, and Behre, *ibid.*, **67**, 267 (1926); Benedict, Newton, and Dakin, *Science*, **64**, 602 (1926), give the structural formula of thiasine.

<sup>25</sup> Folin and Trimble, *J. Biol. Chem.*, **60**, 473 (1924).

<sup>26</sup> Folin, *J. Biol. Chem.*, **54**, 153 (1922); Folin and Wu, *ibid.*, **38**, 459 (1919).

DETERMINATION OF AMINO-ACID NITROGEN<sup>27</sup>

The method is based upon the color reaction between amino-acids and  $\beta$ -naphthaquinone-sulfonic acid. This reaction takes place very slowly in neutral solutions. The stronger the alkalinity, up to a certain point, the more rapidly the color develops. The different amino-acids do not show the same acceleration, however, to definite increases in alkalinity, and the chromophoric reagent (the quinone) is more rapidly destroyed when the alkalinity is increased, giving rise to deep-colored decomposition products. The proper degree of alkalinity, therefore, must be carefully maintained.

In solution  $\beta$ -naphthaquinone-sulfonic acid gradually decomposes and the solution becomes darker in the course of a few hours, particularly if it is not kept in the dark. On this account only freshly prepared solutions should be used.

The sodium acetate in the acetic acid-acetate solution serves two purposes: (1) it increases the color of the quinone-amino-acid derivative and (2) retards very much the onset of turbidity due to the liberation of sulfur from the added sodium thiosulfate. Both of these results are due to the repressed hydrogen-ion concentration of the acetic acid caused by the added excess of acetate ions.

The purpose of the sodium thiosulfate solution is to destroy the surplus quinone remaining after the full color obtainable from the amino-acids has developed. It destroys the surplus color of the quinone and under the conditions of the procedure has no effect on the colored quinone-amino-acid derivative, at least during the first hour or two. Nor do the colored solutions become turbid, within the first 2-hour period, because of liberated sulfur.

**Reagents.**

1. Acetic acid-acetate solution. Mix 50 cc. of 50 per cent acetic acid with an equal volume of 5 per cent sodium acetate solution.
2. Sodium thiosulfate, 4 per cent.
3. Sodium carbonate. Dilute 50 cc. of an approximately saturated solution to 500 cc. Titrate this solution against 20 cc. of 0.1 N hydrochloric acid, using methyl red as an indicator. Dilute the solution so that 8.5 cc. of it are equivalent to 20 cc. of 0.1 N acid. This gives a carbonate solution of about 1 per cent strength.

<sup>27</sup> O. Folin, J. Biol. Chem. **51**, 377 (1922).

4. Amino-acid reagent. Use a freshly prepared 0.5 per cent solution of the sodium salt of  $\beta$ -naphthaquinone-sulphonic acid. For a method of preparing the quinone, see the original paper.

5. Standard amino-acid solution. Prepare a solution containing 0.07 mg. of nitrogen per cubic centimeter. Glycine, tyrosine, leucine, or phenylalanine may be used. Glycine may be purified, if necessary, by recrystallizing it from its water solution by adding 0.5 to 1 volume of alcohol. Make up the amino-acid standard in 0.1 N HCl containing 0.2 per cent sodium benzoate.

**Procedure.**—Transfer 10 cc. of the blood filtrate to a test tube graduated at 25 cc. To a similar tube add 1 cc. of the standard amino-acid solution and 8 cc. of water. Add a drop of phenolphthalein solution to each tube. Next add 1 cc. of the sodium carbonate solution to the standard, and then to the blood filtrate, drop by drop, until enough of the carbonate has been added (usually 6 to 8 drops) to give the same pink color as that in the standard. Then add to both standard and unknown 2 cc. of freshly prepared amino-acid reagent, mix, and let the solutions stand overnight in a dark place. The following day add to each tube 2 cc. of the acetic acid-acetate solution and 2 cc. of 4 per cent sodium thiosulfate solution. This decolorizes the excess of reagent. Dilute both solutions to the 25 cc. mark, mix well, and compare their colors in a colorimeter.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 7 = \begin{cases} \text{mg. of amino-acid nitrogen per 100 cc.} \\ \text{of blood.} \end{cases}$$

## CHAPTER XLVII

### BLOOD ANALYSIS—Continued

SUGAR, CHOLESTEROL, LIPOID PHOSPHORUS (LECITHIN), BILE PIGMENT, BILE SALTS, PROTEINS, PHENOLS, HEMOGLOBIN, IRON, HYDROGEN ION, ETC.

#### DETERMINATION OF SUGAR BY FOLIN'S MODIFICATION OF THE FOLIN-WU METHOD <sup>1</sup>

THE protein-free blood filtrate is heated with an alkaline copper solution in a special tube to prevent atmospheric reoxidation of the cuprous oxide formed by the reducing action of the sugar. The cuprous oxide thus formed is treated with a phosphomolybdic acid reagent and the resulting intense blue-colored solution is compared with a standard. The phosphomolybdic acid reagent contains sodium tungstate because there is sodium tungstate in the Folin-Wu blood filtrates, and tungstates modify somewhat the shade of blue obtained in the reaction.

This modified method employs more carefully adjusted reagents than the original Folin-Wu method and gives normal values for blood glucose which are lower and probably more nearly accurate.

#### Reagents.

1. Alkaline copper tartrate solution. Dissolve the following in about 700 cc. of distilled water: 12 grams of Merck's sodium tartrate (or 15 grams of Rochelle salt), 7 grams of anhydrous sodium carbonate and 20 grams of sodium bicarbonate. Pour the solution into a liter volumetric flask and add to it 200 cc. of water containing 5 grams of copper sulfate. Dilute to the mark and thoroughly mix.

2. Acid molybdate reagent. Dissolve 150 grams of sodium molybdate,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , in 300 cc. of distilled water. Filter the solution through a quantitative filter paper, allowing the filtrate to run into a liter volumetric flask, and finally wash the filter with 75 cc. of

<sup>1</sup> Folin, J. Biol. Chem., **67**, 357 (1926); Folin and Wu, *ibid.*, **41**, 367 (1920).

water. Add 2 or 3 drops of bromine to the solution, shake till the bromine has dissolved, and let stand for an hour to insure complete oxidation by the hypobromite. Next add, with shaking, 225 cc. of 85 per cent phosphoric acid. The excess bromine is liberated and colors the solution yellow. Then add 150 cc. of a solution of sulfuric acid prepared by adding 1 volume of the concentrated acid to 3 volumes of water and cooling. Aerate for about half an hour to remove the excess bromine, add 75 cc. of 99 per cent acetic acid, dilute to a liter and mix.

3. Stock solution of glucose. Use a 1.0 per cent glucose solution made up in saturated benzoic acid. Dilute standards are made with water, and a few drops of toluene or formalin are added as a preservative.

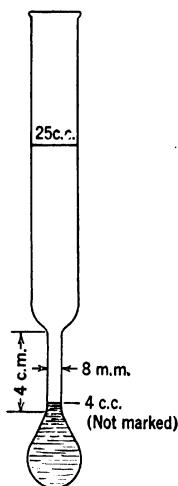


FIG. 57.—Folin and Wu Sugar Tube. [J. Biol. Chem., 41, 372 (1920)].

**Procedure.**—In this procedure the blood filtrate must be about neutral. Ten cubic centimeters of the filtrate should require about 0.2 cc. of 0.1 N NaOH for neutralization. In case the filtrate is not neutral, add a drop of phenolphthalein to 2 cc. of it and then add 0.1 N NaOH, drop by drop, till the pink endpoint is obtained. The same number of drops of 0.1 N NaOH are then added to the sugar tubes before adding the blood filtrates. If the laboratory conditions are kept constant, the required number of drops of 0.1 N NaOH may be added to the sugar tubes at once, thus avoiding the necessity of repeating the preliminary titration. Add 2 cc. of the neutral or nearly neutral blood filtrate to a Folin and Wu sugar tube (see Fig. 57), and

to another add 2 cc. of standard glucose solution. Next add 2 cc. of the alkaline copper tartrate solution to each tube, heat for 10 minutes in a boiling water-bath, cool, and add 2 cc. of the acid molybdate reagent to each tube. As soon as the carbon dioxide apparently ceases to escape (about 1 minute), dilute the solutions to 25 cc., mix, and compare their colors.

**Calculation.**—If a 0.2 mg. glucose standard is used the calculation becomes:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 100 = \text{mg. of glucose per 100 cc. of blood.}$$

Where a 0.4 mg. glucose standard is employed, multiply the ratio of the readings by 200 instead of 100.

**DETERMINATION OF SUGAR BY BENEDICT'S MODIFICATION  
OF THE FOLIN-WU METHOD<sup>1</sup>**

**Reagents.**

1. Alkaline copper reagent. Two hundred grams of sodium citrate and 60 grams of anhydrous sodium carbonate are dissolved in about 800 cc. of distilled water. Then add, with stirring, 100 cc. of a solution containing 6.5 grams of pure copper sulfate, 9 grams of ammonium chloride, dilute to a liter, and mix. Place a 100 cc. portion of the solution in a small bottle and add 2.5 to 3.0 grams of sodium sulfite. The reagent is then ready for use and will keep 3 or 4 weeks. If only occasional determinations are to be made, omit adding sulfite to the reagent but add 5 drops of a 20 per cent sodium sulfite solution to each sugar tube just before adding the reagents.

2. Tungstic acid reagent. Place 600 cc. of water in a liter flask, add 100 grams of pure sodium tungstate, and shake till solution is complete. Then add 50 grams of pure arsenic pentoxide, 25 cc. of 85 per cent phosphoric acid, 20 cc. of hydrochloric acid, sp. gr. 1.19, and boil the solution for 20 minutes. Cool, add 40 grams of sodium chloride, 45 cc. of hydrochloric acid, sp. gr. 1.19, 60 cc. of formalin, and shake till solution is complete. Dilute to a liter and mix.

3. Standard glucose solution. Use pure aqueous solutions of glucose preserved with toluene.

**Procedure.**—Accurately measure 2 cc. of the 1 : 10 tungstic acid filtrate into a Folin and Wu sugar tube and then add 2 cc. of the alkaline copper reagent. Mix the solutions and place the tube in boiling water. The ammonium salt holds the reduced copper in solution. After heating for 5 minutes, remove the tube and put it in cold water. To the cooled solution add 2 cc. of the tungstic acid reagent. The color develops at once. Let stand 1 or 2 minutes, dilute to the 25 cc. mark, thoroughly mix, and match against a standard solution similarly treated.

**Calculation.**—Make the calculation as directed above.

<sup>1</sup> J. Biol. Chem., 68, 759 (1926).

**DETERMINATION OF SUGAR BY BENEDICT'S PICRIC ACID METHOD<sup>3</sup>**

This method is based upon the red to brown color obtained by heating glucose with picric acid and sodium carbonate. These colors were first described by Braun.<sup>4</sup> He states that glucose, fructose, and lactose give these colors and that picramic acid is formed. Later observers were Jaffé,<sup>5</sup> Johnson,<sup>6</sup> and Chapman.<sup>7</sup> All these investigators employed *caustic alkalis* instead of sodium carbonate and, hence, the colors obtained resulted not only from the reduction of picric acid by the sugars, but also from the caramelization of the sugars by the caustic alkali.<sup>8</sup> "This latter effect is entirely avoided in the use of sodium carbonate."<sup>9</sup>

In blood analysis the proteins are removed by precipitation with picric acid, and, since the latter is one of the reagents of the color reaction, it need not be removed from the protein-free filtrate. The trace of creatinine present in blood is probably not sufficient to affect the color value.

**Reagents.**

1. Picrate-picric acid reagent. Dissolve 36 grams of dry powdered picric acid in 500 cc. of 1 per cent sodium hydroxide and about 400 cc. of hot water. As soon as solution is complete, cool, pour into a liter flask, dilute to the mark, and mix.

2. Sodium carbonate, 20 per cent. Dissolve 200 grams of anhydrous carbonate in a liter of water.

3. Standard sugar solution. Prepare simultaneously along with the unknown, by treating 0.64 mg. of pure glucose with 4 cc. of water, 4 cc. of the picrate-picric acid reagent and 1 cc. of 20 per cent sodium carbonate solution. The mixture is heated for 10 minutes in boiling water and then diluted to 12.5 cc.

4. Permanent standards. Solutions of picramic acid or potassium dichromate may be used as permanent standards. The dichro-

<sup>3</sup> Benedict, J. Biol. Chem., **34**, 203 (1918); Lewis and Benedict, *ibid.*, **20**, 61 (1915); cf. Pearce, *ibid.*, **22**, 525 (1915); and Myers and Bailey, *ibid.*, **24**, 147 (1916).

<sup>4</sup> Z. anal. Chem., **4**, 185 (1865); Chem. Zentr., **1866**, 219; *ibid.*, **1874**, 825.

<sup>5</sup> Z. physiol. Chem., **10**, 391 (1886).

<sup>6</sup> Pharm. J. and Trans., **54**, 24.

<sup>7</sup> Analyst, **34**, 475.

<sup>8</sup> Lancet, **25**, Sept., **1844**; Chem. Zentr., **1847**, 623; Chem. Ztg., **1901**, Rep., **209**; Münch. med. Wochschr., **1906**, 1309.

<sup>9</sup> W. M. Dehn and F. A. Hartman, J. Am. Chem. Soc., **36**, 403 (1914).

mate solution does not match the unknown exactly, but will give satisfactory results.

(a) *Picramic Acid Standard*.—To prepare a stock solution, dissolve 100 mg. of pure picramic acid and 200 mg. of sodium carbonate in about 500 cc. of water in a liter flask, dilute to a liter, and mix thoroughly. Treat 126 cc. of the stock solution with 1 cc. of 20 per cent sodium carbonate solution and 15 cc. of the picrate-picric acid reagent, dilute to 300 cc., and mix thoroughly. The resulting solution exactly matches in color that obtained by treating 0.64 mg. of glucose as directed in (3) and diluting to 12.5 cc.

(b) *Potassium Dichromate Standard*.—Dissolve 800 mg. of pure potassium dichromate in about 500 cc. of water, dilute to a liter, and thoroughly mix.

**Procedure.**—Draw 4 or 5 cc. of blood into a test tube containing a little powdered potassium oxalate to prevent clotting. Withdraw 2 cc. of the blood by means of an Ostwald pipette and transfer it to a 25 cc. graduated flask, or to a large test tube marked at 12.5 and at 25 cc. Rinse the pipette twice with distilled water, adding the washings to the blood. Shake the contents of the flask or test tube a minute or two to insure thorough mixing and a consequent laking or hemolysis of the blood. Now add, to the 25 cc. mark, the picrate-picric acid reagent, using a few drops of alcohol to dispel foam, if necessary, and thoroughly mix by shaking. After a minute or two (or longer) pour the mixture upon a dry filter, and collect the clear filtrate in a dry beaker. Measure exactly 8 cc. of the filtrate into a large test tube graduated at 12.5 cc. and at 25 cc., and add 1 cc. of 20 per cent sodium carbonate solution. Plug the tube with cotton and immerse it in boiling water for 10 minutes. (See Note 1.) Remove the tube and cool its contents under the water tap. Dilute to 12.5 cc. or to 25 cc., depending upon the depth of color. At any time within half an hour, compare the solution in a colorimeter with a suitable standard. (See Note 2.)

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 100 = \text{mg. glucose per 100 cc. of blood.}$$

If the unknown is set at 20, then five times the reading of the standard equals the glucose concentration in milligrams per 100 cc. Where the final dilution is made to 25 cc. instead of 12.5 cc., the final figure is, of course, multiplied by 2.



**Notes.**

1. Longer heating of the blood filtrate up to half an hour makes no change in the color.

2. Occasionally the final filtrates in this or other picric acid methods develop a little turbidity during heating. Unless such turbidity is fairly marked, it is of no account. When desired, the final colored solution may be filtered through a small folded filter into the colorimeter cup.

**DETERMINATION OF CHOLESTEROL AND FATTY ACIDS BY THE METHOD OF BLOOR, PELKAN, AND ALLEN <sup>10</sup>**

Hot alcohol-ether solution is used to extract the lipoids from the blood plasma. The extract is then saponified, the cholesterol extracted with chloroform and determined colorimetrically, and the soaps extracted with hot alcohol. The fatty acids (in the form of soaps) are determined nephelometrically by the turbid solution obtained upon acidifying the soap solution. For the nephelometric procedure. see Volume II.

**Reagents.**

1. Sulfuric acid, sp. gr. 1.84. and 1 : 3.
2. Acetic anhydride.
3. Alcohol (redistilled).
4. Ether (redistilled).

5. Chloroform. The chloroform used must be neutral in reaction and free from moisture and alcohol.

6. Sodium hydroxide. Make from metallic sodium by exposing the metal, in a closed vessel containing distilled water at room temperature, over a receiver to catch the hydroxide which drips off the metal. The action is slow, but the apparatus requires little attention and a strong pure hydroxide is obtained.

7. Standard cholesterol solution. This is a solution of cholesterol in chloroform containing from 0.5 to 1 mg. of cholesterol in 5 cc., depending on the cholesterol content of the blood which is being measured. For most purposes a standard containing 0.50 mg. of cholesterol in 5 cc. of solution will be found suitable. For convenience in weighing the cholesterol, a standard twenty times the strength of the final one is prepared, and this is diluted as needed.

<sup>10</sup> J. Biol. Chem., **52**, 191 (1922).

**Procedure: Extraction and Saponification.**—Five cubic centimeters of blood plasma are measured into a 100 cc. flask containing about 75 cc. of a mixture of 3 parts alcohol and 1 part ether (both redistilled). The plasma is made to enter in a slow stream of drops and the liquid in the flask is kept rotating rapidly to prevent the formation of large aggregates of precipitate. At once, or after standing till a convenient time, the flask is immersed in boiling water with frequent and strong rotation (to prevent superheating) until the liquid begins to boil, then cooled to room temperature, made up to volume, mixed, and filtered. For the determination, a volume (10 to 20 cc.), containing about 2 mg. of fatty acid, is measured into a small Erlenmeyer flask (50 to 100 cc.) of Kavalier glass (Pyrex is less suitable for boiling caustic alkalis. See Table XLV, page 720) 0.1 cc. of concentrated NaOH made from sodium is added, and the mixture evaporated on the water-bath. When the volume of liquid has been reduced to a few drops the flask should be rotated or shaken occasionally so as to distribute the liquid evenly over the bottom (but not over the sides). The drying is then continued until only 2 or 3 drops of liquid remain and the odor of alcohol is entirely gone. The alkali is then *partially* neutralized by the addition of 0.1 cc. of dilute sulfuric acid (1 volume concentrated acid, 3 volumes water), and the liquid well mixed and distributed over the bottom of the flask as before. The drying is then continued on the water-bath until the residue is dry and all the moisture has disappeared from the sides of the flask. The process of drying is a very important step in the method, since the separation is not quantitative if the drying is either carried too far (in which case some of the cholesterol cannot be recovered by the cold treatment), or not far enough (when a part of the soap or fatty acids is extracted with the cholesterol). The amount of acid added should be somewhat less than enough to neutralize the alkali, since otherwise fatty acids would be set free and dissolve in the chloroform. For the same reason the added acid should be well mixed with the residue in the flask so as to insure its complete neutralization. If there is not enough liquid in the flask to allow complete mixing, a drop or two of distilled water should be added. The reason for the addition of acid is two-fold; first, the acid prevents destruction of cholesterol by the strong alkali (for, contrary to the statements in the literature, cholesterol is altered—at least as far as its color-producing properties are concerned—by heating with strong alkali); and, second, by the formation of the crys-

talline sodium sulfate the residue is made porous so that the solvents penetrate readily. The heating should be carried through all its stages on a water-bath and not on an electric hot-plate, since it has been found impossible to prevent overheating on the latter.

*Separation and Determination of Cholesterol.*—After cooling, 10 cc. of chloroform are added and the flask is allowed to stand for 10 minutes; it may be shaken occasionally so that the solvent may reach any material adhering to the sides. The chloroform extract is poured through a  $5\frac{1}{2}$  cm. hardened filter into another small flask and the extraction twice repeated with 5 cc. of chloroform. If the drying and distribution of the salt have been carefully carried out, very little of the salt will break loose from the bottom of the flask during the chloroform extraction, and the fatty acids will be quantitatively retained. The combined chloroform extract is then evaporated down to 2 or 3 cc., poured into a 10 cc. glass-stoppered, graduated cylinder, made up to 5 cc. with chloroform washings from the flask, and the cholesterol then determined by the use of the Liebermann-Burchard reaction as follows: To the contents of the graduated cylinder made up to 5 cc. are added 1 cc. of acetic anhydride and 0.1 cc. of pure concentrated sulfuric acid, the cylinder is stoppered, and the whole well mixed. The cylinder is allowed to stand for 15 minutes at a temperature of 20 to 22° C., exposed to the same light by which readings are later to be made. (The color is sensitive to light and this precaution is necessary in order to avoid changes in tint during the reading.) It is then transferred to the colorimeter cup and compared with a suitable standard similarly prepared from pure cholesterol. The standard cholesterol for this purpose should contain ordinarily 0.5 mg. of cholesterol in 5 cc. of chloroform.

#### DETERMINATION OF CHOLESTEROL BY THE METHOD OF MYERS AND WARDELL MODIFIED <sup>11</sup>

The blood plasma or serum is mixed with plaster of Paris, dried, and extracted with chloroform. Acetic anhydride and concentrated sulfuric acid are added to the chloroform extract and, after standing 15 minutes to allow the color to develop, the solution is compared with a standard.

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.

<sup>11</sup> V. C. Myers and E. L. Wardell, *J. Biol. Chem.*, **36**, 147 (1918).

2. Acetic anhydride.

3. Chloroform. Use redistilled chloroform.

4. Standard cholesterol solution. Prepare a stock solution by dissolving 0.160 gram of pure cholesterol in 100 cc. of redistilled chloroform. Dilute 5 cc. of the stock solution to 100 cc. with chloroform. Ten cubic centimeters of this solution contain 0.8 mg. of cholesterol.

5. Permanent standards. Standardize aqueous solutions of naphthol green B. This dye excellently matches the cholesterol color and appears to be permanent.

**Procedure.**—One cubic centimeter of blood, plasma or serum, is pipetted into a porcelain crucible or small beaker containing 4 to 5 grams of plaster of Paris, stirred, and dried, preferably in a drying oven. It is now emptied into a small extraction shell (4 cm. long) and then inserted in a short test tube ( $2.5 \times 6$  cm.), in the bottom of which are a number of small holes (Fig. 58). This is now attached to a large cork on a small reflux condenser and the tube and cork are inserted in the neck of a 150 cc. extraction flask containing about 20 to 25 cc. of chloroform. Extraction is continued for 30 minutes on an electric hot-plate, the chloroform made up to some suitable volume, such as 15 cc., filtered if necessary, and colorimetric estimation carried out as follows: 5 cc. of the chloroform extract are pipetted into a dry test tube, and 2 cc. of acetic anhydride and 0.1 cc. of concentrated sulfuric acid (best with 0.1 cc. pipette) are added. After thorough mixing, the solution is placed in the dark for exactly 10 minutes to allow the color to develop, and then compared with a standard solution of cholesterol in chloroform treated in exactly the same way or a permanent standard of naphthol green B. If a Duboscq colorimeter is used, the cups should be remounted in plaster of Paris instead of balsam.

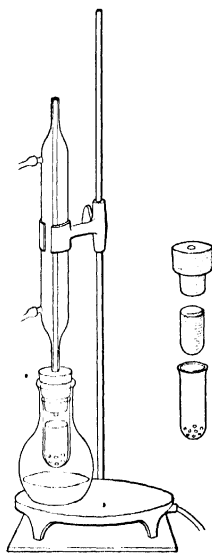


FIG. 58.—[Myers and Wardell, J. Biol. Chem., **36**, 150 (1918)].

Figure 59 illustrates a very neat and compact apparatus<sup>12</sup> which may be used instead of the one shown in Fig. 58. Place the extraction thimble in the siphon cup (a perforated porcelain cup may be used), add chloroform to the flask (Pyrex) and carry out the extraction while cold water flows through the condensing coil.

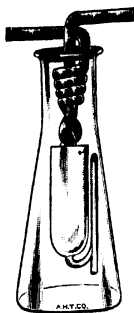


FIG. 59.

**Note.**—In order to get the proper temperature for color development in warm weather, it is advisable either to keep the reagents in a cool place or to insert the tubes in water during the development of the color.

#### DETERMINATION OF LIPOID PHOSPHORUS (LECITHIN) BY THE METHODS OF BLOOR, AND BENEDICT AND THEIS<sup>13</sup>

The lipoids are extracted from the blood with an alcohol-ether mixture, oxidized by a nitric acid-sulfuric acid mixture, and the lipid phosphoric acid determined colorimetrically by the method of Benedict and Theis for inorganic phosphate given on page 353.

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Nitric acid, sp. gr. 1.42.
3. Sodium hydroxide. Use a concentrated, CO<sub>2</sub>-free, solution of sodium hydroxide.
4. Alcohol-ether solution. Use only redistilled alcohol and ether. Mix in the proportion of 3 parts of alcohol to 1 part of ether.
5. Sucrose solution, 1 per cent.
6. For additional reagents, see page 353.

**Procedure.**—Place 20 cc. of the alcohol-ether solution in a 25 cc. graduated flask, and add to it 1 cc. of well-mixed blood. The blood is added in a slow stream of drops and the liquid in the flask is kept rotating fairly rapidly in order to prevent the formation of large aggre-

<sup>12</sup> Obtained from A. H. Thomas Co., Philadelphia. Suggested for cholesterol extractions by W. H. Stoner; cf. P. B. Hawk and O. Bergeim, *Practical Physiological Chemistry*, 9th ed., p. 392. P. Blakiston's Son and Co., Philadelphia, 1926.

<sup>13</sup> Bloor, J. Biol. Chem., **36**, 33 (1918); Benedict and Theis, *ibid.*, **61**, 63 (1924); see also Oser and Karr, *Arch. Int. Med.*, **36**, 507 (1925).

gates of precipitate which are difficult to extract. The flask is then immersed in boiling water with frequent and vigorous rotation (to prevent superheating) until the liquid begins to boil. Cool to room temperature, dilute to 25 cc. with the alcohol-ether solution, mix, and filter. To carry out the oxidation, introduce 5 cc. of the filtrate into a large Pyrex test tube graduated at 5 cc., immerse the tube in boiling water, and evaporate the contents to dryness. Add 0.5 cc. of a mixture of equal parts of sulfuric acid, sp. gr. 1.84, and nitric acid, sp. gr. 1.42, and thoroughly mix with the residue. Digest over a microburner, taking care not to overheat. Water and oxides of nitrogen are driven off first, and finally sulfuric acid fumes. Reduce the flame and continue heating till the brown fumes reappear. Cool for 2 minutes, add 1 or 2 drops of 1 per cent sucrose solution to produce a charred mixture, and heat again. The solution should become clear, but if a brown or yellow color remains after heating for half a minute add a trace of nitric acid and continue boiling. Cool, add 2 cc. of distilled water, rinsing down the sides of the tube, and approximately neutralize by adding a previously determined amount of concentrated,  $\text{CO}_2$ -free, sodium hydroxide solution. Cool to room temperature, dilute with distilled water to the 5 cc. mark, and complete the analysis as directed in the Benedict and Theis method for inorganic phosphorus on page 353, using the same standard phosphorus solution.

#### *Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.025 \times \frac{100}{0.2} = \text{mg. lipoid phosphorus per 100 cc. of blood.}$$

If the unknown is set at 12.5 mm., the result is obtained directly by reading the standard. "Lecithin" contains approximately 4 per cent of phosphorus.

#### DETERMINATION OF BILE PIGMENT IN SERUM. ICTERIC INDEX

The intensity of the yellow pigmentation of serum is matched against a standard potassium dichromate solution.

#### **Reagents.**

1. Sodium chloride, 0.9 per cent.
2. Standard dichromate solution. Use a 0.01 per cent potassium

dichromate solution, containing 2 drops of concentrated sulfuric acid per 500 cc. Keep the solution in a dark-brown bottle or in the dark. Preston<sup>14</sup> recommends the use of a colored glass ("Uran") disk as standard. These disks may be obtained from the Klett Mfg. Co., New York City.

**Procedure.**—Separate the serum from about 5 cc. of freshly drawn, unhemolyzed blood, and dilute 1 cc. of the serum with 0.9 per cent sodium chloride solution until the color approximately matches that of the standard dichromate solution. The dilution may be conveniently made in an ordinary graduated cylinder. Transfer the solutions to a colorimeter and compare their colors. The Bock-Benedict and Hellige-Leitz instruments are well suited for small volumes of liquids.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \text{dilution} = \text{Icteric Index.}^{15}$$

*Example:* If the unknown is set at 10, and 1 cc. of serum diluted to 8 cc. gives a reading on the standard of 6.5, the *icteric index* is 5.2.

#### THE VAN DEN BERGH TEST FOR BILE PIGMENT IN SERUM<sup>16</sup>

This test has won considerable favor among clinicians. It is based upon the red color obtained by treating serum with a diazotizing reagent, the reaction with bilirubin being taken as an index of the type and extent of bilirubinemia, depending upon the rate of appearance of color and its depth. For details of the procedure and interpretation of results, see Hawk and Bergeim, *Practical Physiological Chemistry*, 9th ed., page 396, P. Blakiston's Son and Co., Philadelphia, 1926.

#### DETERMINATION OF BILE SALTS

For a colorimetric method for the determination of bile salts in blood, see P. Szilárd, *Biochem. Z.*, **159**, 325 (1925); *ibid.*, **173**, 440 (1926).

<sup>14</sup> J. Lab. Clin. Med., **11**, 879 (1926).

<sup>15</sup> Meulengracht, *Deut. Arch. klin. Med.*, **132**, 285 (1920).

<sup>16</sup> *Presse Méd.*, **29**, 441 (1921).

## DETERMINATION OF PHENOLS

Moir<sup>17</sup> has described a sensitive test for phenols using diazotized *p*-nitroaniline base. This reagent has been employed by Theis and Benedict<sup>18</sup> for the quantitative colorimetric determination of phenols in the blood. An orange to red coloration is obtained, and the reaction is sufficiently delicate to be employed for phenol determination in blood filtrates, provided the blood is diluted only 1 to 5 instead of 1 to 10 as in the regular Folin-Wu procedure. The reagent tends to become turbid with blood filtrates, but this can be prevented by the addition of a colloid (gum acacia). The reaction takes place in solutions of very weak acidity. This condition is obtained by adding sodium acetate prior to adding the reagent. Uric acid reacts so feebly with the nitroaniline reagent (giving about one-twentieth as much color as an equal weight of phenol) that its removal is unnecessary. The diazotized reagent must be prepared fresh daily.

## Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Sodium carbonate, 20 per cent.
3. Sodium acetate, 50 per cent.
4. Gum acacia, 1 per cent.
5. Diazotized nitroaniline reagent. Dissolve 1.5 grams of *p*-nitroaniline base in 500 cc. of water containing 40 cc. of hydrochloric acid, sp. gr. 1.19. Prepare the diazotized reagent fresh daily by adding 0.75 cc. of 10 per cent sodium nitrite solution to 25 cc. of the *p*-nitroaniline solution.
6. Standard phenol solution. Make a phenol solution in 0.1 N hydrochloric acid that contains about 1 mg. of crystallized phenol per cubic centimeter. Transfer 25 cc. of this solution to a 250 cc. flask, add 50 cc. of 0.1 N sodium hydroxide, heat to 65° C., add 25 cc. of 0.1 N iodine solution, stopper the flask, and let stand at room temperature for half an hour. Then add 5 cc. of hydrochloric acid, sp. gr. 1.19, and titrate the excess of iodine with 0.1 N sodium thiosulfate. Each cubic centimeter of 0.1 N iodine corresponds to 1.567 mg. of phenol. On the basis of the titration, dilute a portion of the phenol solution so

<sup>17</sup> J. South African Inst., **5**, 8 (1922).

<sup>18</sup> J. Biol. Chem., **61**, 67 (1924); see also Rakestraw, *ibid.*, **50**, 109 (1923).



that 1 cc. contains 0.1 mg. of phenol. This dilution is made every few weeks and the final dilution (10 cc. = 0.025 mg.) is made daily.

The 0.025 mg. standard reads with a satisfactory degree of accuracy against solutions containing 0.05 to 0.015 mg. of phenol.

**Procedure.**—The blood is precipitated as in the Folin-Wu procedure except that 2 volumes of water are added instead of 7. To 10 cc. of the 1 : 5 filtrate add 1 cc. of 1 per cent gum acacia solution, 1 cc. of 50 per cent sodium acetate solution, and 1 cc. of the diazotized nitroaniline reagent. After 1 minute add 2 cc. of 20 per cent sodium carbonate solution. A bright orange-red color is obtained. Compare the solution with a similarly treated standard phenol solution containing 0.025 mg. of phenol in 10 cc.

To determine total phenols (free and conjugated), 10 cc. of the 1:5 blood filtrate are put into a test tube with 0.25 cc. of hydrochloric acid, sp. gr. 1.19, and heated for 10 minutes in a boiling water-bath. Cool the solution and neutralize with sodium hydroxide solution. The same amount of acid and alkali are also added to the standard and to the unheated filtrate. The determination is completed as directed in the preceding paragraph.

#### Notes.

1. Blood contains from 1 to 2 mg. of free phenols per 100 cc. Conjugated phenols may occur in small quantity (0.1–0.2 mg. per 100 cc. blood) in some bloods, but are not demonstrable in all bloods.

2. Figures obtained<sup>19</sup> by this method on twenty bloods average 0.4 mg. per 100 cc. lower than the figures of the Rakestraw<sup>20</sup> method on the same bloods.

#### DETERMINATION OF HEMOGLOBIN BY THE NEWCOMER METHOD

**Bausch & Lomb Hemoglobinometer.**—Figure 60. This hemoglobinometer is an adaptation of the Dubosq type of colorimeter. It is a precision instrument which reads directly the per cent hemoglobin to 5 per cent with the least possible manipulation, and is as small and convenient as is consistent with accurate readings.

In addition to the detailed description which follows, there are certain general considerations which should be clearly set forth. The

<sup>19</sup> R. C. Theis and S. R. Benedict, *J. Biol. Chem.*, **61**, 70 (1924).

<sup>20</sup> *J. Biol. Chem.*, **58**, 109 (1923).

basis on which the instrument is constructed is the making of a photometric match between a standard filter on one side and the diluted blood sample on the other. The filter against which the match is made is of special yellow glass. The selection of the filter is the result of extensive investigations upon the absorptive characteristics of the hemoglobin. The filter itself was selected after the examination of over one thousand samples of glass. For a discussion of the theory of this hemoglobinometer, see H. S. Newcomer, J. Biol. Chem., **37**, 465 (1919).

The 100 per cent point of the scale is determined by the normal value of Williamson,<sup>21</sup> which is 16.92 grams per 100 cc. This value, 16.92, is the average of the hemoglobin determinations of 919 normal individuals, and has been arbitrarily chosen as the base for the scale. Reference to the chart and descriptive matter, pages 486-487, will show how this average normal value is affected by age and sex. The chart is based

on the same series of measurements from which the normal value is obtained.

**Description of Instrument.**—*The body* of the instrument is of aluminum, to which are firmly attached the integral parts of the equipment, made as small and light as possible while still retaining firmness and rigidity.

*The rack and pinion* actuating the cup table is of the precision microscope type, both cups being operated by the same pinion.

*The prism system*, *P*, is of the double rhomboid type, the inner

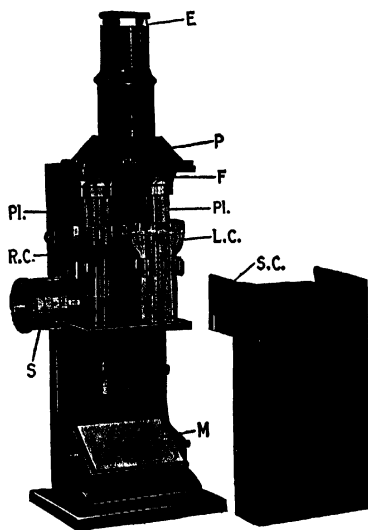


FIG. 60.—Hemoglobinometer. E, eyepiece; P, prism system; F, filter; Pl, plungers; R.C., right cup; L.C., left cup; S, scale; S.C., slip cover; M, mirror.

<sup>21</sup> Arch. Int. Med., **18**, 505 (1916).

reflecting surfaces of both rhomboids being silvered. The dividing line of the field is formed by the sharp edge of a silver reflecting surface. Since the dividing line becomes practically invisible, very delicate and accurate settings are assured. In keeping with the idea that the instrument should be as compact as possible, the prism has been designed in a smaller form than that used in the Duboscq colorimeters.

*The observing eyepiece, E*, consists of a collective and eye lens, so diaphragmed at the eyepoint that all disturbing reflections are removed.

*The cups, RC and IC*, are of clear glass with their bases of optical glass fused firmly upon them. The tops are flared to prevent any overflow of liquid even when the plunger is brought in contact with the bottom of the cup. Their actual depth is 47.5 mm.; the fluid depth is 37.5 mm.

*The plungers, Pl*, are of hollow tubing of the same glass as the cups. The bases are fused on to the tubes and, in addition, a second disk is cemented to the upper end for the purpose of keeping out dirt and dust which might otherwise enter.

*The filter, F*, as pointed out above, is of a special yellow glass chosen for its spectral properties. It is introduced into the left-hand light path in a recess provided at the upper end of the plunger mount. The thickness of the filter is controlled to 0.01 mm., making unnecessary any correction for filter thickness. This filter is not balanced by a similar disk of clear glass on the right side, since it has been found that the best approximation to the absorption curve of normal hemoglobin is secured when the loss of light by surface reflection is added to the selective absorption of the filter.

*The scale, S*, is calibrated to the normal value of 16.92 grams per 100 cc. To the pinion which operates both cups simultaneously is attached a rotating grooved drum on which are engraved the hemoglobin values in steps of 5 per cent. The liquid depth (in per cent hemoglobin) is shown by an indicator which moves in and out with the rotation of the drum. The use of this drum has made it possible to lengthen very materially the individual units. The shortest division (between 145 and 150 per cent) covers about  $1\frac{1}{2}$  mm. or about  $6\frac{1}{2}$  degrees of arc. The longest division (between 40 and 45 per cent) covers about 19 mm. The length of these divisions, which individually cover 5 per cent, makes it possible to estimate easily the hemoglobin value to within 1 per cent. In addition to the feature of accuracy, the

drum offers an excellent reading device. With only a slight shift of the eye from its position of observation of the field, the drum and its indicator are clearly seen, and the reading made directly.

In order to secure maximum illumination, which will be at the same time diffuse and even, a second surface mirror, *M*, having its first surface ground, is used.

Each instrument is equipped with a special pipette holding 5 cc. This pipette is calibrated to give dilutions of 250 to 1 or 500 to 1. The normal dilution, and the one from which the scale is calibrated, is 500 to 1. The second dilution is to be used only for bloods of very low hemoglobin content.

There is provided also a slip cover which can be used to protect the cups from extraneous light and the instrument from dirt when it is not in use.

**Procedure.**—The blood from a puncture wound is drawn to the 10 mark (10 cu. mm.) in the pipette and the remainder filled with the diluent which is a solution of 1 per cent hydrochloric acid. With this dilution the instrument will read as low as 40 per cent hemoglobin. Readings of blood from individuals having a lower hemoglobin content may be made by doubling the amount of blood used and dividing the resultant colorimeter reading by 2. To make such a dilution of double strength, blood should be drawn to the 20 mark and the pipette filled with diluent.

Place clear water in the left-hand cup, *LC*, and the contents of the pipette in the right-hand cup, *RC*. See that both rest squarely on the carriage. Bring the bottom of the cups in contact with the plungers, *Pl*, to force out any air bubbles. This may also be accomplished by tilting the instrument. Finally, balance the two color fields until they have the same color density.

The yellow solution obtained by dilution of the blood with 1 per cent hydrochloric acid increases in color density with the lapse of time. The instrument is calibrated to read correctly when the solution has reached its final color depth. When readings are not made too soon after dilution, this difference, for practical purposes, may be neglected. If more accurate results are desired, the reading may be corrected by a simple calculation based on the proportion of the rate of change in color density to the lapse of time. The absolute reading at any given time is equal to the observed reading plus a percentage of the reading.

This percentage is the quotient of 40 divided by the lapse of time between the making of the dilution and the reading, thus:

$$\text{Absolute reading} = \text{observed reading} + \frac{40}{100T} (\text{observed reading}).$$

*Table for Time Correction.*—To correct the hemoglobinometer reading, add to the reading a figure in the table, the choice of the figure depending on the time since dilution of the blood and the reading of the hemoglobinometer.

TABLE XLII

Reading, Per Cent	Minutes Since Dilution			
	10	15	20	40
35-44	1.5	1.0	1.0	0.5
45-59	2.0	1.5	1.0	0.5
60-69	2.5	1.5	1.25	0.5
70-80	3.0	2.0	1.5	1.0
81-91	3.5	2.5	1.5	1.0
91-109	4.0	3.0	2.0	1.0
110-119	4.5	3.0	2.0	1.0
120-134	5.0	3.0	2.5	1.0
135-145	6.0	4.0	3.0	1.5

**Chart of Hemoglobin Values. Birth to Old Age.**—Figure 61 is drawn from data on the hemoglobin of 919 normal individuals, published by Williamson.<sup>22</sup> The two curves represent, respectively, the mean values of the hemoglobin content of blood of normal males and females from birth to old age. A line is drawn across the figure at a level corresponding to 16.92 grams hemoglobin per 100 cc. of whole blood. This figure is the average for males during the age period sixteen to sixty years. It is arbitrarily chosen as the 100 per cent of the hemoglobin scale, and the rest of the abscissas are drawn accordingly.

A line drawn at a level corresponding to 15.53 grams (91.8 per cent) indicates the average for females during the same age interval.

From an examination of the curves one can see the way in which the expected hemoglobin will deviate from this 100 per cent figure,

<sup>22</sup> Arch. Int. Med., 18, 505 (1916)

according to age and sex. It is normal for individuals to range as much as 1 gram (6 per cent) above or below the values given by these curves.

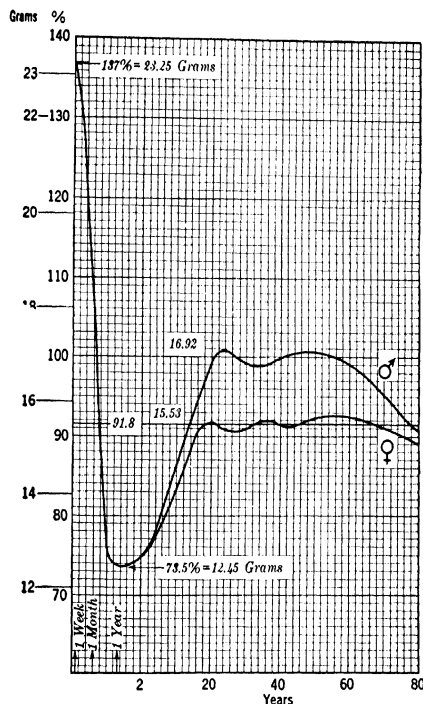


FIG. 61.—Newcomer's Chart of Hemoglobin Values—Birth to Old Age—Based upon Williamson's Data.

#### DETERMINATION OF HEMOGLOBIN BY THE ACID HEMATIN METHOD OF COHEN AND SMITH

This method is based upon measuring the depth of color of acid hematin resulting from the dilution of blood in dilute hydrochloric acid. The procedure and notes have been taken from the work of Cohen and Smith.<sup>23</sup>

<sup>23</sup> J. Biol. Chem., **39**, 489 (1919).

**Reagents.**

1. Hydrochloric acid, 0.1 N.

2. Standard acid hematin. A quantity of blood (usually 50 cc.) is obtained, carefully defibrinated, and then strained through gauze. The oxygen capacity of this blood is then determined by the Van Slyke method.<sup>24</sup> Accepting the Haldane figure of 18.5 volumes per cent for the oxygen capacity of normal blood (corresponding to approximately 14 grams of hemoglobin per 100 cc.), the blood is diluted with 0.1 N HCl so as to make a 20 per cent solution of a blood with an oxygen capacity of 18.5 volumes per cent. That is, if the blood has an oxygen capacity of 18.5 volumes per cent, 20 cc. of it are diluted to 100 cc. with 0.1 N HCl; if the oxygen capacity is 22 volumes per cent, then 16.8 cc.  $\left(\frac{18.5 \times 20}{22}\right)$  of it are diluted to 100 cc. with the acid. The 20 per cent solution of blood thus obtained is well mixed and stored in a glass-stoppered bottle, preferably in a cool spot away from the light. This constitutes the stock solution from which the comparison standard is made. Such a stock solution will not deteriorate for at least 3 months, provided contamination by molds is avoided. Sahli<sup>25</sup> suggests saturating the acid with chloroform to keep molds from developing in the solution. No other unusual precautions for the preservation of this solution seem to be needed; but before using, it should be thoroughly shaken.

*The comparison standard* for use in the colorimeter is made by diluting 5 cc. of the stock solution to 100 cc. with 0.1 N HCl to make a 1 per cent standard; or 2.5 cc. of the stock solution to 100 cc. for a 0.5 per cent standard. Where routine determinations are to be made, it is desirable to have the standards made fresh at least once a week.

Where determinations of the oxygen capacity of the blood are not readily available, one can make the stock solution from crystallized hemoglobin.

**Procedure.**—From a freely flowing source of blood, 0.02 cc. is measured by means of a calibrated Sahli pipette into 6 cc. of 0.1 N hydrochloric acid. The blood pipette is rinsed out by drawing the acid solution into it several times. Blood very low in hemoglobin may require a double sample, i.e., 0.04 cc. of blood in 6 cc. of acid, in order to give a

<sup>24</sup> J. Biol. Chem., **33**, 127 (1918).

<sup>25</sup> H. Sahli, *Lehrbuch der Klinischen Untersuchungs-Methoden*, 6th ed., ii, p. 293. Leipzig, 1915.

dilution having the most satisfactory color for comparison with the standard. After the sample is added to the acid, the mixture must be allowed to stand, preferably in a warm place, for at least 10 minutes for the full color to develop. Readings taken sooner will be too low.

In cold weather, hemolysis and color development take place more slowly, and the application of gentle heat will hasten the process. As a routine procedure, immersion of the tube in a warm water-bath is recommended. The color comparison may be made in either the Autenrieth-Hellige or the Duboscq colorimeter with a standard acid hematin solution. The average of at least several readings is taken. The calculation is simple and is described for each instrument as follows:

*Standard and Calculation for the Duboscq Colorimeter.*—The standard for comparison is a 0.5 per cent blood solution, which is set at 10 upon the Duboscq scale. Hence the per cent hemoglobin =  $\frac{1.5 \times 10 \times 100}{\text{reading}}$ .

It is desirable to make an actual calibration of the instrument for the solutions to be examined.

*Standard and Calculation for the Autenrieth-Hellige-Leitz Wedge Colorimeter.* (See Fig. 62.)—The standard for comparison is a 1.0 per cent blood solution. Experience has pointed to the necessity for different concentrations of standard solutions in each kind of instrument in order to secure most satisfactory color comparisons. The scale on the Autenrieth colorimeter may be inaccurately placed. There is another source of error that cannot be corrected by a mere resetting of the scale. The glass wedge containing the standard is not mathematically perfect; therefore, for accurate work, this wedge containing the 1 per cent standard should be calibrated against solutions of known amounts of blood in 0.1 N HCl contained in the small cup. Thus, a curve may be constructed from which may be read at once the percentage hemoglobin corresponding to a given reading on the scale. This calibration takes a short time, and holds good for that wedge and instrument as long as other conditions are maintained.

For the conditions given here, if the instrument were perfectly constructed, the calculation would be  $\frac{3.0 \times 10 \times 100}{\text{reading}}$  = per cent hemoglobin, but this relation holds for only a portion of the scale (between 3.0 and 8.5). Sahli<sup>26</sup> claims the color development reaches a maxi-

<sup>26</sup> *Loc. cit.*



mum in 1 minute when the proportions he recommends are used: 0.02 cc. of blood in 0.20 cc. of 0.1 N.HCl.

Meyer and Butterfield<sup>27</sup> found considerable delay in the full development of the color of the acid hematin. Cohen and Smith observed that the temperature of the solution, as well as the concentration of acid, greatly affects the speed of color development, and therefore resorted, when necessary, to warming the solution. An

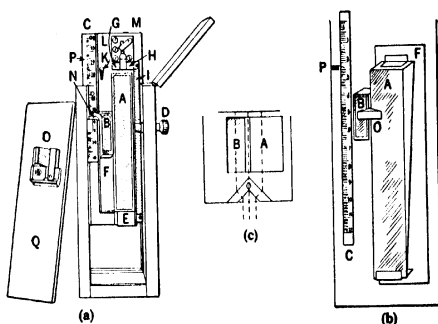


FIG. 62.—*A*, glass wedge for the "known"; *B*, glass trough for the "unknown"; *C*, scale; *D*, pinion for manipulating the rack for displacing the wedge relative to the glass trough; *E*, projected support for holding the glass wedge; *F*, ground glass plate for diffusing the light; *G* and *H*, metal holder which fits over the stopper of the glass wedge and holds the glass wedge in place; *I*, rack which pinion *D* manipulates; *K*, clasp which holds the ground glass plate in place; *L*, wooden slider on which the various parts are mounted; *M*, clamp which manipulates the holder *G-H* for screwing the glass wedge in place; *N*, metal holder which acts as support for the glass trough *B* and which support can be removed; *O*, double prism (Helmholtz); *P*, indicator against which the scale displaces and wherever an equality in the color of the solution is determined the scale portion opposite the indicator represents the reading of percentage; *Q*, wooden board acting as support for the double prism and as cover for the front portion of the colorimeter.

interval of 10 minutes seems to be sufficient for the development of practically the maximum depth of color.

Newcomer<sup>28</sup> studied the rate of color development in acid hematin and found that after 10 minutes the color development was 96 per cent; after 20 minutes, 98 per cent; and after 40 minutes, 99 per cent of the maximum. He gives a useful formula for calculating the color deficiency:  $xy = -40$ , where  $x$  is the time in minutes, and  $y$  the percentage of color deficiency. The constant,  $-40$ , was apparently

<sup>27</sup>Arch. Int. Med., 14, 94 (1914).

<sup>28</sup>J. Biol. Chem., 37, 465 (1919).

derived for room temperature, and is not applicable to a wide range of temperatures, according to the experience of Cohen and Smith.

#### DETERMINATION OF IRON IN BIOLOGICAL MATERIAL

This is a thiocyanate method developed by Walker for the determination of iron in food products and other biological materials.<sup>29</sup> He studied both the thiocyanate and the ferrocyanide methods and found that the former is generally preferable.

##### Reagents.

1. Nitric acid, sp. gr. 1.42.
2. Hydrogen peroxide.
3. Potassium thiocyanate, 10 per cent.
4. Standard iron solution. Dissolve 0.7000 gram of pure ferrous ammonium sulfate,  $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , in about 100 cc. of distilled water. Add 5 cc. of sulfuric acid, sp. gr. 1.84, warm and add potassium permanganate solution until oxidation is complete. Cool, dilute to a liter, and mix thoroughly. One cubic centimeter contains 0.1 mg. of Fe.

**Procedure.**—Accurately weigh about a 5 gram sample into a porcelain evaporating dish and ignite in an electric furnace. Cool, add 10 cc. of distilled water, 3 cc. of nitric acid, sp. gr. 1.42, and filter through a small quantitative filter paper. Add 2 cc. of hydrogen peroxide to the filtrate, let stand for 1 minute, and then add 5 cc. of 10 per cent potassium thiocyanate solution. Dilute to 50 cc. and match against a standard prepared by treating 1 cc. of the standard iron solution in the same way except that 2 cc. of nitric acid are used instead of 3 cc.

##### Notes.

1. The extra 1 cc. of nitric acid used for the sample is to neutralize the alkalinity due to the ash.
2. If the phosphate content is small, the ash may be dissolved in hydrochloric acid; for moderate amounts (not over 0.05 gram  $\text{H}_3\text{PO}_4$ ) nitric acid should be used; for large amounts of phosphate as compared with iron (as in milk) use the method of Elvehjem and Hart.<sup>30</sup>

<sup>29</sup> Analyst, **50**, 278 (1925).

<sup>30</sup> J. Biol. Chem., **67**, 49 (1926).

3. The thiocyanate method is unsatisfactory in the presence of Ag, Hg, Co,  $\text{H}_2\text{C}_2\text{O}_4$ , and HF.

#### DETERMINATION OF HYDROGEN ION

##### CULLEN'S METHOD AS MODIFIED BY HAWKINS.<sup>31</sup>

For a discussion of the general principles underlying the colorimetric method of determining hydrogen-ion concentration, see Chapter XIX.

##### Reagents.

1. Sodium chloride, 0.9 per cent.
2. Saline indicator solution.<sup>32</sup> Add 2.1 cc. of 0.03 per cent phenol red (phenolsulfonephthalein) solution to 100 cc. of 0.9 per cent sodium chloride solution, mix, and adjust approximately to pH 7.4 by adding 0.02 N sodium hydroxide with a fine glass rod which has been dipped into the alkali.
3. Sörensen's standard phosphate solutions.<sup>32</sup> Prepare solutions ranging in steps of 0.05 pH from pH 7.00 to 7.80. For directions, see Clark, *The Determination of Hydrogen Ions*, 2d ed., Williams & Wilkins Co., 1922.

**Procedure.**—Thirty drops of a 0.03 per cent solution of phenol red are added to 50 cc. of a 0.9 per cent solution of sodium chloride and adjusted to pH 7.3. Five cubic centimeter portions of this solution are placed in tubes with a diameter of 16 mm. and covered with paraffin oil.

The blood is drawn from the heart of a guinea pig, or a vein of a human subject, directly into a 1 cc. pipette graduated to hundredths, by attaching a needle with a short rubber tube to the pipette.

The needle and rubber are then detached and 0.25 cc. of the blood is run under the oil into one of the tubes containing the saline indicator solution. The blood and solution are thoroughly mixed by stirring carefully with a clean glass rod.

The tube is centrifuged for 10 minutes, completely throwing down the red corpuscles, and is then placed in a comparator block and the pH determined by matching to the nearest color standard and applying

<sup>31</sup> Cullen, *J. Biol. Chem.*, **52**, 501 (1922); Hawkins, *ibid.*, **57**, 493 (1923).

<sup>32</sup> Indicators and standard buffer salts, both dry and in prepared solutions, may be obtained from the LaMotte Chemical Products Co., Baltimore, Md., or Hynson, Westcott, and Dunning, Baltimore, Md.

corrections as described by Cullen. The standard color tubes are 16 mm. in diameter and contain 5 cc. of Sörenson's standard phosphate solutions ranging in steps of 0.05  $pH$  from  $pH$  7.00 to 7.80.

Use a saline plasma tube as a control to compensate the standard for the slight color and turbidity of the plasma. For the arrangement of the tubes in the comparator block, see page 211.

#### Notes.

1. The color standards slowly fade and must be checked once a week against a freshly prepared standard. Keep the color standards in the dark when they are not in use.

2. Be sure to test the  $pH$  of the distilled water. It usually has a  $pH$  between 6.2 and 6.5 and should give no red color with either phenol red or methyl red. Use both indicators. Also, test the oil by shaking it with water containing phenol red and methyl red. The water must remain neutral.

3. All apparatus must be thoroughly rinsed with redistilled water and dried. To compensate for the protein and salt errors in the colorimetric determination, an empirical correction,  $-0.23$  (Cullen's correction), is applied. This gives values which agree more closely with those determined electrometrically at  $38^{\circ}C$ .  $pH_{38^{\circ}} = \text{colorimetric } pH_t + 0.01 (t^{\circ} - 20) - 0.23$ , where  $t^{\circ}$  is the observed temperature.

Under pathological conditions there is considerable variation in the Cullen correction.<sup>33</sup>

4. Normal blood ranges in  $pH$  from 7.30 to 7.50. In pathological cases the range so far observed is from  $pH$  6.95 to 7.80.

5. For a description of a multiple wedge colorimeter for bicolorimetric work, see V. C. Myers, *J. Biol. Chem.* **54**, 675 (1922). This instrument is manufactured by E. Leitz, Inc., New York.

#### METHOD OF McCLENDON, RUSSELL, AND TRACY<sup>34</sup>

This method for the determination of the hydrogen-ion concentration in blood plasma makes use of a Duboscq colorimeter. The determination requires an indicator that shows change only in intensity of color with degree of dissociation and not a mixture of two

<sup>33</sup> Austin, Stadie, and Robinson, *J. Biol. Chem.*, **60**, 505 (1925); see also, Hastings and Sendroy, *ibid.*, **61**, 695 (1924).

<sup>34</sup> *J. Biol. Chem.*, **70**, 705 (1926).

colors such as is the case with phenol red. There are only a few such indicators with the sensitive portion of their range within the physiological limits of blood  $pH$ . Of these, cyanine (quinoline blue) is very strongly affected by proteins or some other constituent of plasma and, hence, does not permit an accurate matching of color. Paranitrophenol is a fairly strong precipitant of the plasma proteins but may be used. The best indicator found by McClendon, Russell, and Tracy is ortho-chrom-T, which they obtained from the Farbwerke vorm. Meister Lucius und Brüning, Hoechst. This indicator, which is used as a photosensitizer, is easily oxidized by air, but is not appreciably affected during the short period necessary to make a  $pH$  determination.

#### Reagents.

1. Potassium oxalate, 30 per cent.
2. Sodium carbonate, 0.01 N.
3. Barium hydroxide, 0.05 N.
4. Paraffin oil.
5. Ortho-chrom-T indicator solution. Dissolve 0.1 gram of the indicator in 10 cc. of alcohol and mix with 90 cc. of distilled water. Keep in a bottle with a dropping pipette.



FIG. 63.

**Apparatus.**—In order to prevent loss of  $CO_2$  from the diluted blood plasma, two methods have been tried. Perhaps the best is the use of the cup shown in Fig. 63 which has a fused-on glass bottom and a removable cover, which, when seated, gives a depth of fluid of exactly 20 mm. Indicator in distilled water is poured into the cup and the plasma is introduced through a long fine point of a pipette into the bottom of the cup, causing the indicator solution to overflow. The cover is then quickly seated without air bubbles, and the cup grasped with a towel and rotated violently to stir up the contents. Tight fitting of the glass cover prevents the loss of  $CO_2$ .

Another method is the use of the cup with side neck shown in Fig. 64. This cup has the top and bottom fused on, the distance between

them being exactly 20 mm., and has a side neck for filling. 4.5 cc. of distilled water containing the indicator are introduced, the top level being up in the side neck and a layer of paraffin oil floated over



FIG. 64.

it. The plasma is introduced by inserting the finely drawn out tip of a pipette down the side neck; after introducing the plasma the contents are stirred with the pipette point or a needle.

For micro determinations, the cup with side neck shown in Fig. 65 has been used with the Buerker colorimeter; the top and bottom are fused on, being exactly 10 mm. apart. This cup was made by the Bausch and Lomb Optical Company. The original cups of the Buerker colorimeter have not the bottoms fused on and therefore they are liable to leak. Also the arrangement is awkward for introducing plasma into the bottom of the cup and getting the cover on without air bubbles.



FIG. 65.

The arrangement of the cup with side neck in the biological colorimeter is shown in Fig. 66. The cup with side neck is placed below the plunger cup on the left hand side, the plunger cup being filled with distilled water. On the right hand side a cup similar to Fig. 63 is placed below the plunger cup, this cup shown in Fig. 63 being known as the accessory cup and containing a mixture of distilled water and plasma to give the same degree of cloudiness and yellow color as the plasma on the left hand side containing the indicator.

The plunger cup on the right hand side contains indicator in 0.01 N sodium carbonate solution in which it is approximately 100 per cent dissociated.<sup>35</sup>

The optical system is now theoretically symmetrical, but in practice is found not to be so. It seems that the indicator increases the colloidal aggregation of the proteins of the plasma and hence the left hand side shows more cloud than the right-hand side. In order to avoid this, barium hydroxide is added to the plunger cup on the right-

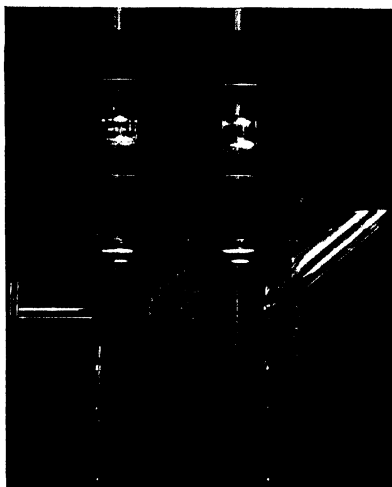


FIG. 66.

hand side and immediately precipitated by action of the sodium carbonate, forming a cloud.

**Procedure.**—The blood is drawn by means of an oiled Luer syringe and immediately transferred to a centrifuge tube previously prepared. The centrifuge tube contains enough 30 per cent potassium oxalate solution to make 0.1 per cent in the blood (for animals double quantity is used). A layer of paraffin oil is floated over the potassium oxalate solution. The needle of the syringe is inserted into the potassium

<sup>35</sup>The arrangement in Fig. 66 may be modified in order to avoid the cup with side neck by substituting for it another accessory cup as shown in Fig. 63.

oxalate solution and the blood is transferred to the centrifuge tube and immediately centrifuged. A 1 cc. pipette, graduated in 0.01 of a cc., with a very long finely drawn out tip is inserted into the plasma and the plasma sucked up into it.

Since the plasma will dilute the indicator, the indicator solution in the cup with the side neck is made stronger than that in the sodium carbonate solution used as a standard. The plunger cup on the left is filled with water. In the plunger cup on the right-hand side are placed 5 cc. of sodium carbonate solution plus 2 drops of 0.05 N  $\text{Ba}(\text{OH})_2$  solution and 3 drops of the indicator. In the cup with side neck on the left-hand side are placed 4.5 cc. of distilled water plus 3 drops of indicator, and oil is floated over it. Then the tip of the pipette containing the plasma is introduced and 0.5 cc. of plasma is allowed to enter, and then stirred. Since the pipette is narrow, the portion of the plasma that has come in contact with the air is not used and does not diffuse into the portion that is used. In the accessory cup on the right-hand side is placed a mixture of 5 cc. of water plus 0.5 cc. of plasma, and the readings are made. Since the side neck cup on the left-hand side is 20 mm. deep, the readings in mm. on the right-hand side divided by 0.2 would give the percentage dissociation of the indicator. This is then found on the vertical scale in Fig. 67 and the horizontal line run to the diagonal marked *O.C.T.* The point of intersection is traced vertically and the pH read off.

This method was standardized with ox serum in the Clark hydrogen electrode at 27°. If there is any change in temperature or salt content or any variation in the indicator, it should be restandardized by finding one point on Fig. 67 and drawing a diagonal parallel to the one given for 27°.

Since oil is troublesome to clean from the side neck of the cup, it is more convenient to use the cup shown in Fig. 63. This cup as made for McClendon, *et al.*, holds exactly 3 cc. when the cover is seated. It is filled to overflowing with a mixture of 4.5 cc. of water plus 3 drops of the indicator and 0.3 cc. of the plasma is introduced carefully *at the bottom* avoiding any mixing until the cover is seated. The cover is quickly seated and the cup is grasped in a towel and rotated until mixed, when it is placed below the plunger cup on the left hand side of the colorimeter. The rest of the procedure is the same as the above.

*Note.*—Dr. Sevringhaus of the University of Wisconsin Medical School found that barium carbonate precipitated so rapidly that he



substituted a gum mastic colloidal solution, made by dropping an alcoholic solution of gum mastic into the aqueous solution.<sup>36</sup>

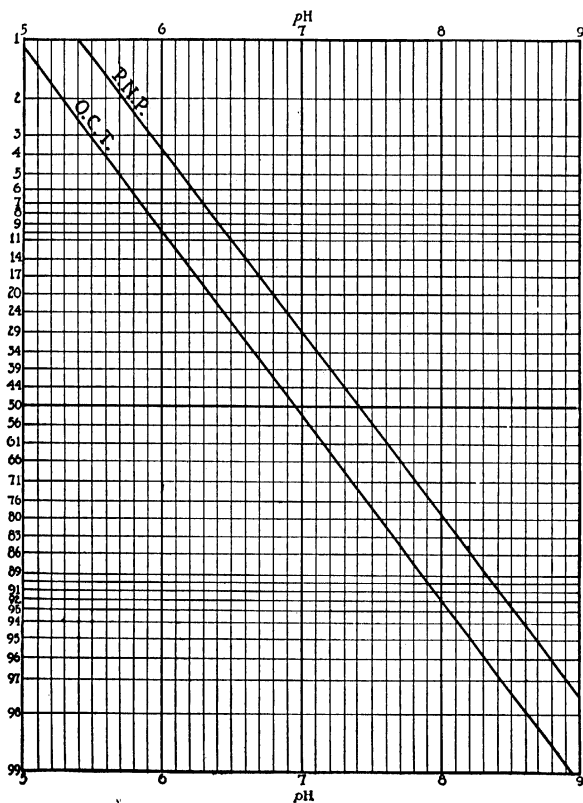


FIG. 67.

#### DETERMINATION OF CALCIUM

See pages 139, 143 and 146.

#### DETERMINATION OF MAGNESIUM

See page 264.

<sup>36</sup> Private communication from Dr. J. F. McClendon.

**DETERMINATION OF INORGANIC PHOSPHATE**

See pages 346, 348, and 353.

**DETERMINATION OF CHLORIDE**

See page 159.

**DETERMINATION OF SILICON IN TISSUE**

See page 366.

For the nephelometric determination of a number of substances in biological material, see Volume II.

## CHAPTER XLVIII

### URINE ANALYSIS

#### TOTAL NITROGEN, UREA, AMMONIA AND AMINO-ACID NITROGEN

##### DETERMINATION OF TOTAL NITROGEN IN URINE

FOLIN AND FARMER<sup>1</sup> have developed a microchemical method for the determination of total nitrogen in urine, based on the Kjeldahl-Gunning process for decomposing nitrogenous materials and on the methods of Nessler and of Folin for the determination of ammonia. Rapidity in every stage of the procedure is obtained by reducing the amount of urine taken for an analysis. Only 1 cc. of *diluted* urine is used. This is digested with concentrated sulfuric acid, potassium sulfate, and a little copper sulfate as catalyzer. The potassium sulfate is converted by the acid into potassium acid sulfate, which serves to raise the boiling-point of the sulfuric acid, and thus hastens the digestion. The reactions involved are, in general, illustrated in the determination of non-protein nitrogen in blood. (See p. 446.) The ammonia thus formed is liberated with an excess of alkali and carried over into an acid solution by means of an air current. The resulting solution is then treated with Nessler's reagent and the color produced compared with that of a standard solution of an ammonium salt similarly treated.

Bock and Benedict<sup>2</sup> have modified the Folin-Farmer procedure by distilling the ammonia instead of removing it by aspiration. They consider the distillation procedure more accurate than aspiration. Folir. and others, however, find the aspiration procedure gives satisfactory results.

McCrackan, Passamaneck and Harman<sup>3</sup> recommend a simple apparatus that may be used for steam distillation, or aspiration, and that eliminates bumping and back suction.

<sup>1</sup> J. Biol. Chem., **11**, 493 (1912).

<sup>2</sup> J. Biol. Chem., **20**, 47 (1915).

<sup>3</sup> J. Lab. Clin. Med., **11**, 678 (1926).

An Erlenmeyer flask, *A*, Fig. 68, clamped in position on wire gauze, and filled a third or less full of distilled water containing one or two drops of concentrated sulfuric acid, is used as a steam generator. It has a safety tube, *B*, at least thirty inches long, which is slightly constricted at its lower end. A test tube is sometimes hung over the upper end to prevent the entrance of dust. A delivery tube, *D*, constricted a little at its lower end, leads through the rubber stoppers, *C* and *E*, from flask, *A*, into a tube or flask attached to rubber stopper, *E*. The delivery tube, *D*, may be bent in such a way as to cause the bottom of the test tube to rest on the edge of the gauze that supports the flask, *A*, making it possible to heat both with one burner. Similarly a second delivery tube, *F*, with a much constricted outlet, leads through rubber stoppers, *E* and *G*, into dilute acid in a tube or volumetric flask, *I*, which is attached to rubber stopper, *G*. The tube, *H*, that leads from *I*, may be attached to a pump, if the apparatus is to be used for aërating. It can be further improved as a

aëration apparatus by making the delivery tube, *F*, from one of Folin's 30 cm. aëration tubes, which ends in a bulb with numerous perforations, and it can be still further improved by causing the air entering flask *A*, to pass through another of these tubes substituted for tube *B*. This can be accomplished by removing *B*, or by capping *B* with a piece of rubber tubing plugged with a glass rod and having the Folin tube pass through a third hole in stopper, *C*, into the dilute acid in flask *A*. In such an arrangement it would only be necessary to remove the rubber cap from one of the tubes leading into flask *A*, to the other, to change an aëration outfit into one for distillation, or vice versa.

Rose <sup>4</sup> has used an apparatus combining the aëration and distillation methods and uses perchloric acid and hydrogen peroxide in the

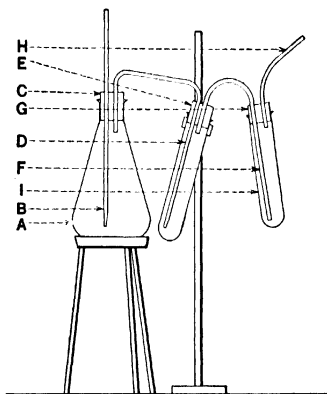


FIG. 68.

<sup>4</sup> J. Biol. Chem., **64**, 253 (1925).

digestion. The method is rapid and accurate. It should be pointed out, however, that the use of too much perchloric acid must be carefully avoided,<sup>5</sup> otherwise a serious loss of nitrogen will result from the oxidation of part of the ammonia to free nitrogen.

In the direct Nesslerization method of Koch and McMeekin<sup>6</sup> the organic matter is destroyed by digestion with sulfuric acid and hydrogen peroxide. The resulting solution is Nesslerized directly and matched against a standard.

Folin and Denis<sup>7</sup> have used a direct Nesslerization method in which a small amount of urine is digested with a phosphoric acid-sulfuric acid-copper sulfate mixture to destroy the organic matter. The resulting solution is treated directly with Nessler's reagent and the color obtained matched against a standard.

#### METHOD A: MICROCHEMICAL METHOD OF FOLIN AND FARMER<sup>8</sup>

##### Reagents.

1. Sulfuric acid, sp. gr. 1.84.
2. Hydrochloric acid, 0.1 N.
3. Sodium hydroxide. Use a saturated solution.
4. Potassium sulfate.
5. Copper sulfate, 5 per cent.
6. Nessler's reagent. (See p. 447.)
7. Standard ammonium sulfate. Use only the pure salt. Pyridine bases are present in all ammonium salts. These bases *titrate* like ammonia but do *not* react with Nessler's reagent.

Pure ammonium sulfate may be prepared by decomposing a good grade of ammonium salt with sodium hydroxide and passing the liberated ammonia into pure sulfuric acid. The salt thus obtained is precipitated by the addition of alcohol, is redissolved in water, again precipitated with alcohol, and finally dried in a desiccator over sulfuric acid.

Pyridine-free ammonium salts can now be obtained on the market.

**Procedure.**—Transfer 5 cc. of urine to a 50 cc. volumetric flask if the specific gravity of the urine is over 1.018, or to a 25 cc. flask if the

<sup>5</sup> B. Mears and R. E. Hussey, *J. Ind. Eng. Chem.*, **13**, 1054 (1921); *cf.* J. H. Yoe, *Ann. chim. anal. chim. appl.* [2], **7**, 193 (1925).

<sup>6</sup> *J. Am. Chem. Soc.*, **46**, 2066 (1924).

<sup>7</sup> *J. Biol. Chem.*, **26**, 486 (1916).

<sup>8</sup> *J. Biol. Chem.*, **11**, 493 (1912).

specific gravity is less than 1.018. The purpose is to dilute the urine so that its nitrogen content is between 0.75 and 1.5 mg. per cubic centimeter. The flask is filled to the mark with ammonia-free water and the solution thoroughly mixed. One cubic centimeter of the diluted urine is then measured into a large Pyrex test tube (20 to 25 mm.  $\times$  200 mm.). Add to this 1 cc. of sulfuric acid, sp. gr. 1.84, 1 gram of potassium sulfate, 1 drop of 5 per cent copper sulfate solution, and a small, clean, quartz pebble or glass bead (to avoid bumping). Boil the mixture over a micro-burner for about 6 minutes, i.e., about 2 minutes after the mixture has become colorless, taking care not to have the flame so high as to unduly heat the test tube above the liquid. Allow the digestion mixture to cool (usually about 3 min.) until it begins to become viscous, but do not let it solidify. Add about 6 cc. of water, at first a few drops at a time, then more rapidly so as to prevent solidification. To this acid solution is then added an excess of sodium hydroxide (3 cc. of saturated solution—see Note 3) and the ammonia thus set free is aspirated by means of a rapid air current into a 100 cc. volumetric flask containing about 20 cc. of ammonia-free water and 2 cc. of 0.1 N hydrochloric acid. Figure 69 illustrates the apparatus for use with compressed air, Fig. 70 that for use with suction. In case suction is employed, collect the ammonia in a large Pyrex test tube containing 2 cc. of 0.1 N hydrochloric acid and about 5 cc. of ammonia-free water, and then transfer the ammonium salt to the volumetric flask with 40 or 50 cc. of ammonia-free water. The air current should be only moderately rapid for the first 2 minutes but thereafter it should be run for 8 minutes at the maximum speed the apparatus will permit.

Dilute the contents of the flask to about 60 cc. with ammonia-free water, and similarly dilute 1 mg. of nitrogen in the form of ammonium sulfate in a second volumetric flask. Nesslerize both solutions as nearly as possible at the same time with 5 cc. of Nessler's reagent, diluted, immediately before using, with about 25 cc. of ammonia-free water. When thus diluted turbidity is avoided. The two flasks are filled to the mark with ammonia-free water, mixed thoroughly, and the solutions matched in a colorimeter.

#### *Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \text{mg. nitrogen in the volume of urine taken.}$$

**Notes.**

1. The color produced in the above reaction does not reach the maximum intensity until the end of about half an hour, but the increase is small and is immaterial to the result when the reagent is added as described, i.e., practically simultaneously to both unknown and standard solutions.

2. The colors are easily matched. Diffused daylight is best but a fairly accurate reading may be made with an electric light provided

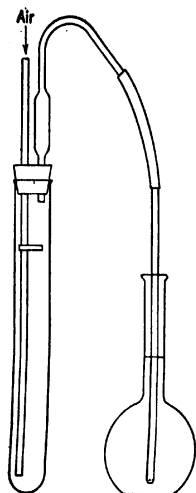


FIG. 69.—Folin and Farmer Aëration Apparatus for use with Compressed Air. [*J. Biol. Chem.*, **11**, 499 (1912)]

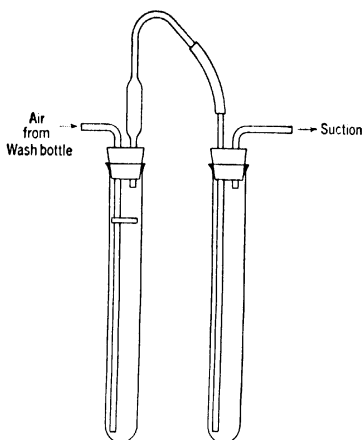


FIG. 70.—Folin and Farmer Aëration Apparatus for use with Suction. [*J. Biol. Chem.*, **11**, 500 (1912)].

a sheet of smooth white paper is interposed between the source of light and the colorimeter. Careful adjustment of the instrument so as to obtain equal illumination in both fields is, of course, necessary.

3. The 3 cc. of saturated sodium hydroxide solution is conveniently added to the digestion mixture by sucking it up into a glass tube which extends to the bottom of the test tube and through which the air is forced through the alkaline mixture. By means of a short rubber tube and a pinchcock the tube is temporarily used as a pipette to transfer the alkali.

METHOD B: BOCK-BENEDICT MODIFICATION OF THE FOLIN-FARMER METHOD<sup>9</sup>

**Reagents.**—Same as those used in Method A.

**Procedure.**—The distillation apparatus is illustrated in Fig. 71.

It consists of a small Liebig condenser made as follows: A piece of glass tubing 30 mm.  $\times$  150 mm. is fitted on each end with a double-holed stopper. A long glass tube with an inside diameter about 5 mm. is inserted through the stoppers and serves as a condensing tube. Two short bent tubes serve as inlet and outlet for the cooling water. The

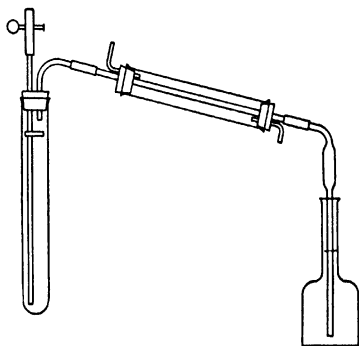


FIG. 71.—Bock and Benedict Distillation Apparatus. [J. Biol. Chem., 20, 57 (1913).]

lower end of the condenser tube is connected with an old pipette (to prevent back suction) which extends into the volumetric flask used as a receiver. The distillation test tube is fitted with a two-hole rubber stopper. Through one hole passes a long tube, reaching almost to the bottom of the test tube, while through the second hole passes a short tube, bent for connecting to the condenser.

The digestion is carried out as directed by Folin and Farmer. After the mixture has partially cooled, add 7 cc. of ammonia-free water. Fill the long tube with 3 cc. of saturated sodium hydroxide solution by suction and close by means of a short rubber tube and a pinchcock. Stopper the test tube by inserting the two-holed rubber stopper and connect the outlet with the condenser. Two cubic centimeters of 0.1 N hydrochloric acid and enough ammonia-free water to cover the connecting tube are put into the receiving flask. The alkali is run into the test tube, the liquids mixed by blowing a few bubbles of air through the apparatus, and the mixture then heated to vigorous boiling (over a large free flame), the distillation being continued until a separation of salts occurs in the test tube and the mixture begins to bump. The

<sup>9</sup> J. Biol. Chem., 20, 47 (1915).



distillation requires about 2 minutes. When distillation is complete, disconnect the test tube from the condenser and wash the latter with a few cubic centimeters of water. Dilute the liquid in the receiving flask and Nesslerize as in the Folin and Farmer method. (See p. 503.)

#### Notes.

1. It is advisable for a person, using this method for the first time, to check his results carefully.
2. Use only ammonia-free reagents.

#### METHOD C: DIRECT NESSLERIZATION METHOD OF KOCH AND McMEEKIN<sup>10</sup>

##### Reagents.

1. Sulfuric acid, 1 : 1.
2. Hydrogen peroxide, 30 per cent. Use Merck's Superoxal or Kahlbaum's Perhydrol. Keep in a cool place and away from direct sunlight. Do not allow the liquid to come in contact with the skin. Its vapors are very irritating to the mucous membrane, and, hence, a pipette must not be used in the usual way to measure out the liquid. Since hydrogen peroxide solutions may contain appreciable amounts of nitrogen, a "blank" determination must be made on each new lot and a correction applied to the analyses if necessary, or the peroxide should be properly redistilled from a slightly acid solution before it is used.
3. Modified Nessler-Folin reagent. Dissolve 22.5 grams of iodine in 20 cc. of water containing 30 grams of potassium iodide. After the solution is complete, add 30 grams of pure metallic mercury, and shake the mixture well, keeping it from becoming *hot* by immersing in tap water from time to time. Continue this until the supernatant liquid has lost all of the yellow color due to iodine. Decant the supernatant aqueous solution and test a few drops with about 1 cc. of a 1 per cent soluble starch solution. Unless the starch test for iodine is obtained, the solution may contain mercurous compounds. To the remaining solution add a few drops of an iodine solution of the same concentration as employed above, until a faint excess of free iodine can be detected by adding a few drops to 1 cc. of the starch solution. Dilute to 200 cc. and mix well.

<sup>10</sup> J. Am. Chem. Soc., **46**, 2066 (1924).

To 975 cc. of an accurately prepared 10 per cent sodium hydroxide solution, now add the entire solution of potassium mercuric iodide prepared above. Mix thoroughly and allow to clear by standing.

This solution is to be used in the proportion of 10 cc. per 100 cc. of solution to be Nesslerized, except in special cases where a great excess of acid is present, as in the direct Nesslerization methods. In such methods one should aim to add an amount of the reagent sufficient to insure the same alkalinity in the unknowns as in the standards.

This modified reagent is an improvement over the Folin-Nessler reagent in that the solution never separates a dark green precipitate of mercurous compounds and also that it is not likely to cause turbidity when added to the ammonia solutions. The reagent is very slightly more sensitive than the original and the Nesslerized solutions remain clear for days.

4. Standard ammonium sulfate solution.—Prepare a solution containing 1 mg. of nitrogen per 5 cc. Use only ammonium sulfate known to be free from pyridine bases. (See p. 502.)

**Procedure.**—Transfer 5 cc. of the well-mixed urine to a 50 cc. graduated flask, dilute to the mark and thoroughly mix. (If the specific gravity of the urine is over 1.018, dilute to 100 cc.) The amount taken for analysis should contain between 0.3 and 1.0 mg. of nitrogen.

Pipette 1 cc. of the diluted urine into a Pyrex test tube ( $20 \times 2.5$  cm.). Add to this 1 cc. of the 1 : 1 sulfuric acid and heat the tube over a free flame (with shaking) or on a sand-bath until the water has been driven off. Then heat over a micro-burner until dense white fumes of sulfuric acid fill the tube. Let cool 20 or 30 seconds, add 1 to 5 drops of 30 per cent hydrogen peroxide solution, and continue heating over the micro-burner. If the liquid remains colorless upon reheating until dense white fumes appear, continue gentle boiling for 2 to 5 minutes. Should the liquid again become discolored, add several drops of the peroxide and repeat the heating. After digestion is complete, cool the liquid, transfer it quantitatively to a 100 cc. volumetric flask, and dilute to about 75 cc. Then add 15 cc. of the modified Nessler-Folin reagent, make up to 100 cc., mix well, and after 5 to 20 minutes compare with a standard. To prepare the standard, mix 1.5 to 5 cc. of the standard ammonium sulfate solution (representing 0.3 to 1.0 mg. N) and 1 cc. of 1 : 1 sulfuric acid solution in a 100 cc. volumetric flask, dilute to about 75 cc., add 15 cc. of the modified

Nessler-Folin reagent, dilute to the mark, and thoroughly mix. Prepare the standard along with the unknown.

*Calculation.*

$$\frac{\text{Reading of standard} \times \text{mg. N in standard}}{\text{Reading of unknown}} = \text{mg. of nitrogen per cubic centimeter of diluted urine used.}$$

**Notes.**

1. A factor which must be carefully controlled is the volume of Nessler reagent added, because the variation in alkalinity affects the intensity of the color. It requires approximately 8.3 cc. of the modified Nessler reagent (containing 8.4 per cent of sodium hydroxide) to neutralize the acid used in the digestion. If now an additional 6.7 cc. of Nessler reagent is added for every 100 cc. of final volume Nesslerized, very nearly the same alkalinity will be obtained in every dilution, that is, a titratable alkalinity equivalent to about 0.56 per cent sodium hydroxide. The most satisfactory results are obtained by always preparing the standards containing 1 cc. of the 1 : 1 sulfuric acid in exactly the same volumes as the unknowns. By so doing the alkalinities and excess of reagent are so nearly identical that theoretical values are easily obtained. When the Nesslerization is conducted in a 50 cc. volume, 12 cc. of the Nessler reagent should be used, and the standard should be similarly prepared in a 50 cc. volume.

2. Koch and McMeekin do not recommend the use of this macro-procedure in place of the usual macro-Kjeldahl because the reagent is costly as compared with potassium and copper sulfates. They do, however, consider the use of hydrogen peroxide very desirable in preventing the troublesome foaming so common with substances high in fats and carbohydrates. Moreover, the method is very rapid.

3. No ammonia is lost in this digestion, even in the presence of chlorides. Also, changes in alkalinity or in concentration of sulfate brought about by differences in length of time of digestion are negligible factors.

**METHOD D: DIRECT NESSLERIZATION METHOD OF FOLIN AND DENIS<sup>11</sup>**

**Reagents.**

1. Sodium hydroxide, 10 per cent.
2. Phosphoric-sulfuric acid-copper sulfate solution. Mix 100 cc.

<sup>11</sup> J. Biol. Chem., 28, 486 (1916).

of sulfuric acid, sp. gr. 1.84, 300 cc. of 85 per cent phosphoric acid, and 15 cc. of 10 per cent copper sulfate solution. Filter if necessary and keep well stoppered. Use only ammonia-free reagents.

3. Nessler reagent. Use a Nessler reagent containing 5 to 6 per cent mercuric potassium iodide,  $\text{HgI}_2 \cdot \text{KI}$ , and 2 per cent sodium hydroxide.

4. Standard ammonium sulfate solution. Prepare a solution containing 1 mg. of nitrogen per 20 cc. Use only pyridine-free ammonium sulfate. (See p. 502.)

**Procedure.**—Use urine diluted so that 1 cc. contains from 0.7 to 1.5 mg. nitrogen. When the urine has a specific gravity of 1.018 or less it should be diluted 1 to 5; when the specific gravity is between 1.018 and 1.030, dilute 1 to 10; and when over 1.030, dilute 1 to 20.

Transfer with an Ostwald pipette 1 cc. of the diluted urine to a large Pyrex test tube, and with an ordinary pipette add 1 cc. of the phosphoric-sulfuric acid-copper sulfate solution. Drop into the tube a clean, quartz pebble or a piece of granite to avoid bumping, and heat over a micro-burner until almost all of the water has been expelled as indicated by the absence of foaming and the appearance of dense white fumes of sulfuric acid. This should require only 2 or 3 minutes. When the fumes appear, cover the test tube with a watch-glass and continue heating at a rate which will keep the tube filled with the acid fumes but with very little escaping. Within 3 minutes from the time the tube is closed the digestion mixture should become clear, and bluish or light green. Continue gentle heating for 30 to 60 seconds. The total heating period must not be less than 2 minutes, counting from the time the tube was closed. Remove the flame, let the tube cool for 2 minutes, add water, and rinse the digestion mixture into a 250 cc. volumetric flask with about 150 cc. of water.

Determine the acid content in 1 cc. of the phosphoric-sulfuric acid mixture by titrating with 10 per cent sodium hydroxide solution, using phenolphthalein as indicator. Then add to the diluted digestion mixture 1.4 times the titrating value obtained, plus 2 cc. for alkalinity. Into another 250 cc. volumetric flask, introduce 20 cc. of the standard ammonium sulfate solution and 1 cc. of the phosphoric-sulfuric acid mixture. Dilute with about 125 cc. of water, add the same amount of sodium hydroxide as added to the unknown and mix well. Then add to each flask 15 cc. of the Nessler reagent, mix quickly, fill to the mark with water, and thoroughly mix.

In case the unknown is turbid, centrifuge or filter (through a small cotton plug) a portion to obtain a crystal-clear solution before Nesslerizing. If the white sediment (silica) after Nesslerizing is mixed with a red deposit the determination was not successful and it must be repeated. Place the unknown and standard in the colorimeter cups and compare their colors.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \frac{\text{mg. of nitrogen in the amount of diluted urine used.}}{\text{mg. of nitrogen in the amount of diluted urine used.}}$$

**Note.**—It is advisable to run a “blank” determination on the reagents.

#### DETERMINATION OF UREA

The urea is converted into ammonium carbonate by means of the enzyme urease, in the presence of a phosphate buffer solution which also catalyzes the conversion. The ammonia may be distilled off, removed by aëration, or determined directly by Nesslerization.

#### METHOD A: DIRECT NESSLERIZATION METHOD OF FOLIN AND YOUNGBURG<sup>12</sup>

**Reagents.**

1. “Permutit.” This material is a synthetic aluminum silicate obtained from the Permutit Company, New York City. Use only 60 to 80 mesh preparation. When added to water it should settle in a few seconds and should not make the water turbid. It may be used repeatedly if after each use it is first washed with water, then with 2 per cent acetic acid solution, and finally with water.

2. Alcoholic urease solution. Place about 3 grams of “Permutit” in a flask, wash once with 2 per cent acetic acid solution and twice with water. Add 5 grams of fine jack bean meal and 100 cc. of 30 per cent alcohol. Shake gently but continuously for about 15 minutes and filter. The filtrate contains practically the whole of the urease and extremely little other materials.

3. Buffer solution. Dissolve 142 grams of  $\text{Na}_2\text{HPO}_4$  and 120

<sup>12</sup> Folin and Youngburg, *J. Biol. Chem.*, **38**, 111 (1919); Youngburg, *ibid.*, **45**, 319 (1921).

grams of  $\text{NaH}_2\text{PO}_4$  (or the equivalent amounts of the crystalline salts) in water and dilute to a liter.

4. Nessler's reagent. See page 447 for the preparation of Nessler's reagent according to Folin and Wu.

5. Standard ammonium sulfate solution. Use only pyridine-free ammonium sulfate. (See p. 502.)

**Procedure.**—Dilute 5 cc. of urine to 50 cc. (10 to 50 if very dilute urine) and mix well. Place 3 to 4 grams of dry "Permutit" in a wide-bottom flask, preferably a 200 or 250 cc. volumetric flask, and add 20 to 25 cc. of the diluted urine. Agitate for 5 minutes. Allow to settle about 30 seconds and then pour through a thin filter paper (known to be free from an appreciable amount of ammonia). If there is no "Permutit dust," the urine may be decanted without filtering. The ammonia is completely removed.

Add 1 cc. of the alcoholic urease solution and 1 drop of the buffer solution to 1 cc. of the diluted urine (dilution usually 1 : 10) in a test tube, and digest in a beaker of warm water (40–55° C.) for 5 minutes or at room temperature for 15 minutes. Then transfer the contents of the tube to a 200 cc. volumetric flask and dilute to about 150 cc. with ammonia-free water. Prepare a standard in another 200 cc. flask by adding 1 mg. of nitrogen in the form of ammonium sulfate and 1 cc. of the urease solution, and dilute to about 150 cc. with ammonia-free water. Then add 20 cc. of Nessler's reagent to each flask, dilute to the mark, mix thoroughly, and compare the two solutions in a colorimeter.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \text{mg. urea N in 0.1 cc. of undiluted urine.}$$

**METHOD B: ROSE AND COLEMAN COLORIMETRIC MODIFICATION OF THE VAN SLYKE AND CULLEN TITRATION METHOD<sup>13</sup>**

**Reagents.**

1. Hydrochloric or sulfuric acid, 0.02 N.
2. Potassium carbonate. Use a saturated solution.
3. Enzyme reagent. Dissolve 2 grams of enzyme preparation, 0.6 gram of  $\text{K}_2\text{HPO}_4$ , and 0.4 gram of  $\text{KH}_2\text{PO}_4$  in 10 cc. of water. Cover

<sup>13</sup> Rose and Coleman, *Biochem. Bull.*, **3**, 411 (1914); Van Slyke and Cullen, *J. Biol. Chem.*, **19**, 141 (1919); see also, Van Slyke and Cullen, *J. Am. Med. Assoc.*, **62**, 1558 (1914); cf. Hawk and Bergeim, *Practical Physiological Chemistry*, 9th ed., p. 722, P. Blakiston's Son and Co., Philadelphia, 1926.

the slightly opalescent solution with toluene. The solution will retain its activity for two weeks.

Satisfactory preparations of urease in tablet or powder form may be obtained from Hynson, Westcott, and Dunning, Baltimore, Md., and from the Arlington Chemical Co., Yonkers, N. Y. For a method of preparing the urease, see Van Slyke and Cullen, *J. Biol. Chem.*, **19**, 211 (1914).

4. Caprylic alcohol.

5. Standard ammonium sulfate solution. Use only the pure salt. (See p. 502.)

**Procedure.**—Figure 72 illustrates the aëration apparatus of Van

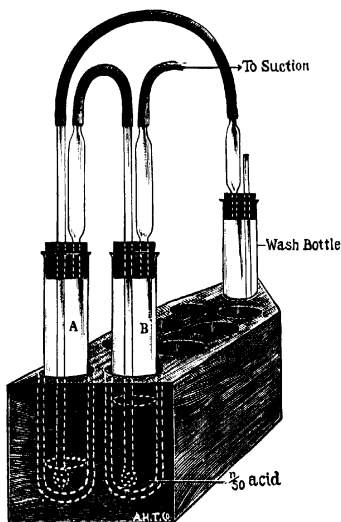


FIG. 72.—Van Slyke and Cullen Aëration Apparatus.

Slyke and Cullen,<sup>14</sup> and their technique is as follows: Dilute 5 cc. of urine to 50 cc. with ammonia-free water. Add to tube *A*, 5 cc. of the diluted urine, 1 drop caprylic alcohol (to prevent frothing), and 1 cc. of the enzyme reagent. Stopper tube *A* as shown in the figure and let stand for 15 minutes to allow complete decomposition of the urea. No harm is done if the solution stands longer, but the time must not be cut shorter unless more enzyme is used. While the enzyme is acting, measure into tube *B* 25 cc. of 0.02 N hydrochloric or sulfuric acid and connect the tubes as shown in the figure. After

the solution in tube *A* has stood 15 minutes, aspirate for one-half minute to remove any free ammonia. Then introduce into tube *A* 5 cc. of a saturated solution of potassium carbonate, close the tube at once, and aspirate until all the ammonia has been carried over into tube *B*. From 5 to 30 minutes will be required to complete the aspira-

<sup>14</sup> *J. Biol. Chem.*, **19**, 217 (1914).

tion, depending upon the type of pump employed. When aspiration is complete, disconnect, taking care to avoid back suction, and Nesslerize the solution in *B* as in the Folin and Farmer method for total nitrogen. (See p. 502). Compare the Nesslerized unknown and standard solutions in a colorimeter.

#### DETERMINATION OF AMMONIA

##### METHOD OF FOLIN AND BELL OR "PERMUTIT" METHOD<sup>15</sup>

The development of this method arose from the need of a suitable absorbent for ammonia to take the place of Merck's purified blood charcoal which was not available in America during the World War. Other charcoals were found unsatisfactory since they failed to abstract creatinine. The substance finally adopted by Folin and Bell is a synthetic mineral, an "aluminate silicate" or "exchange silicate," a zeolite, discovered by Gans.<sup>16</sup> It possesses in a high degree the peculiar absorptive properties characteristic of some of the natural zeolites, and is manufactured and used on a large scale for the "softening" of water and for other industrial purposes. The crude product is sold under the trade name "Permutit" and a purified form is used for ammonia determinations.

The essential mechanical feature of the new reagent is that it is a clean, moderately fine (60 to 80 mesh for ammonia), insoluble powder which gives off no dust or turbid material to water, and settles, like sea sand, from water in the course of a few seconds. By virtue of this novel feature the absorbed ammonia can be separated quickly by decantation of the solution which contained it.

The removal of ammonia by this mineral reagent is not an *adsorptive* phenomenon. "The reagent is a complex insoluble sodium salt containing active, i.e., easily replaceable, sodium, and the absorption of ammonia involves the replacement of a part of this sodium by ammonia. The chemical affinity of the active group in the reagent for ammonia is remarkably strong, so that under suitable conditions the exchange becomes quantitative as far as the ammonia is concerned. The reaction is a reversible one, however, and in the presence of more than small amounts of soluble sodium salts, or other electrolytes, the equilibrium reached does not represent a quantitative

<sup>15</sup> J. Biol. Chem., **29**, 329 (1917).

<sup>16</sup> Jahrb. k. preuss. Geol. Landesanstalt, **26**, 179 (1905); *ibid.*, **27**, 63 (1906).



absorption of the ammonia."<sup>17</sup> Notwithstanding the balanced character of the reaction, there is, however, an adequate margin of safety when working with urine, provided an attempt is not made to work with too much ammonia.

Although the chemical reaction involved in the absorption of ammonia by this reagent is apparently one between a solid and a solution, it should be mentioned that the solid powder contains about 20 per cent of water of hydration and that removal of this water by heat causes a loss in the activity of the reagent. Even gentle dry heat (100° C.) greatly reduces the activity of the reagent.

Ammonia is absorbed best from neutral solutions, but is also absorbed well from weakly acid solutions. The presence of much acid should be avoided since the reagent is soluble in acids. Ammonia is not absorbed in alkaline solutions. Indeed, the usefulness of the reagent depends on the fact that upon the addition of alkali hydroxide the absorbed ammonia is again set free.

Everyone purchasing a supply of the reagent should determine for himself how long the Nesslerization mixture must stand to develop the maximum intensity of color. Folin and Bell found that with their reagent not less than 95 per cent of the theoretical value was obtained in 2 to 3 minutes, and substantially theoretical figures were obtained in 10 to 15 minutes, or less. If the absorbed ammonia is left in the powder over night the liberation of ammonia takes a little longer and the result may be 2 to 3 per cent low.

An important characteristic of the "Permutit" reagent is that it does not appreciably deteriorate with use. After washing away the Nesslerized ammonia and surplus alkali first with water, then with 2 per cent acetic acid, then once more with water, the powder remaining is just as efficient as before for the absorption of more ammonia. In practice, the single charges may be rinsed slightly and poured into a cylinder until a sufficient quantity for recovery has been collected.

### Reagents.

1. Sodium hydroxide, 10 per cent.
2. "Permutit."
3. Nessler's reagent. Use the modified Nessler-Folin reagent. (See p. 506.)

<sup>17</sup> Folin and Bell, *loc. cit.*

**Procedure.**—Place about 2 grams of "Permutit" in a 200 cc. volumetric flask, add about 5 cc. of water (no more), and with an Ostwald pipette introduce 1 or 2 cc. of urine, or with a 5 cc. pipette introduce 5 cc. of previously diluted urine (corresponding to 1 or 2 cc. of the original urine). With urines having a very low ammonia content it may be necessary to use more urine (5 cc.), but insofar as it is practicable, it is better not to use more than 2 cc. and to employ a weaker standard (0.5 mg. of ammonia nitrogen) for the color comparison. Rinse down the added urine by means of 1 to 5 cc. of water, and shake gently but continuously for 5 minutes. Rinse the powder to the bottom of the flask by the addition of 25 to 40 cc. of water and decant. Add water once more and decant. (In the case of urines rich in bile it is advisable to wash once or twice more.) Add a little water to the powder, introduce 5 cc. of 10 per cent sodium hydroxide, mix, and then add more water until the flask is about three-fourths full. Shake for a few seconds and then add 10 cc. of Folin's modified Nessler reagent. Mix, let stand for 10 minutes (or longer), fill to the mark, thoroughly mix and compare in a colorimeter with a 1 mg. nitrogen standard similarly prepared. Unless the standard is deep red and perfectly clear it must be discarded and a fresh one made up.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \frac{\text{mg. of ammonia nitrogen in the amount of urine used.}}{\quad}$$

**DETERMINATION OF AMINO-ACID NITROGEN BY THE METHOD OF FOLIN<sup>18</sup>**

The urine is first treated with "Permutit" to remove ammonia and then with naphthaquinone sulfonic acid, which gives a red color. The colored solution is matched against a standard amino-acid solution.

**Reagents.**

1. Sodium carbonate solution. Dilute 50 cc. of an approximately saturated solution to 500 cc. Titrate 20 cc. of 0.1 N hydrochloric acid with the carbonate solution, using methyl orange as an indicator. Dilute the carbonate solution so that 8.5 cc. are equivalent to 20 cc. of 0.1 N acid. This gives about a 1 per cent carbonate solution.

<sup>18</sup> J. Biol. Chem., 51, 393 (1922).

2. Acetic acid-acetate solution. Mix 100 cc. of 50 per cent acetic acid with 100 cc. of a 5 per cent solution of sodium acetate.

3. Sodium thiosulfate solution. Prepare a 4 per cent solution of the crystalline salt.

4. Amino-acid reagent. Prepare a 0.5 per cent solution of the sodium salt of  $\beta$ -naphthaquinone sulfonic acid. Use a freshly prepared solution. For a method of preparing the quinone, see Folín, J. Biol. Chem., **51**, 386 (1922).

5. Standard amino-acid solution. The following may be used: Glycocoll, leucine, tyrosine, or phenylalanine. Prepare a solution containing 0.1 mg. of nitrogen per cubic centimeter.

**Procedure.**—Dilute 5 or more cc. of urine to a volume of 25 cc. in a 50 cc. Erlenmeyer flask. Add 2 to 3 grams of "Permutit"<sup>19</sup> and agitate very gently, but continuously, for 5 minutes. Decant the supernatant urine into another 50 cc. flask. Again add 2 to 3 grams of "Permutit," and shake as before for 5 minutes. By this double extraction with "Permutit" every trace of ammonia is removed. Decant the supernatant urine into a flask or test tube. It may be a little turbid, but this fact does not interfere with the determination.

To test tubes graduated at 25 cc. add 1, 2, and 3 cc., respectively, of a standard glycocoll solution in 0.1 N hydrochloric acid plus 0.2 per cent of sodium benzoate. This standard solution should contain 0.1 mg. of glycocoll nitrogen per cubic centimeter. To these tubes add 1, 2, and 3 cc., respectively, of the 1 per cent sodium carbonate solution (1 cc. of sodium carbonate for each cubic centimeter of 0.1 N hydrochloric acid present). Dilute the contents of each test tube to a volume of 10 cc.

Transfer 5 cc. of the ammonia-free (usually diluted) urine to another test tube graduated at 25 cc. Add 1 cc. of 0.1 N hydrochloric acid and 1 cc. of the 1 per cent sodium carbonate solution. Dilute to 10 cc. Dissolve 250 mg. of the amino-acid reagent in 50 cc. of water, and add 5 cc. of this solution to each standard and to the unknown urine.

Mix and set in a dark place overnight. It is often advisable to take out the test tubes and inspect them after they have stood 10 to

<sup>19</sup> The special "Permutit" to be used is that prepared by the Permutit Company of New York. The product is now probably obtainable from any of the leading American dealers in chemicals. It is, of course, essential to know that the product is active towards ammonia.

15 minutes. If the test tube containing the urine appears much darker than the darkest standard, as may happen, especially in connection with experiments planned to produce excessive amino-acid excretion, it is, of course, necessary to start another sample of the urine, taking only 1, 2, or 3 cc. and treating it in the same way as the first sample, not omitting to provide for a final volume of 15 cc.

The following day the standard and the unknown or unknowns are first acidified by the addition of 1 cc. of the acetic acid-acetate solution. To each are then added 5 cc. of the 4 per cent sodium thiosulfate solution. The contents of all the tubes are diluted to a volume of 25 cc. and, after mixing, the color of the unknown is read against that of the standard having most nearly the same intensity of color.

For the calculation it is, of course, essential to know which standard is used, and the actual volume of undiluted urine taken for the determination.

## CHAPTER XLIX

### URINE ANALYSIS—Continued

#### CREATININE, CREATINE, URIC ACID, SUGAR, PROTEINS, PHENOLS, MANGANESE, HYDROGEN ION, ETC.

##### DETERMINATION OF CREATININE

WHEN creatinine and an alkaline solution of picric acid are mixed, a red solution is obtained, due to the formation of a red tautomer of creatinine picrate. This reaction is known as Jaffé's reaction. (See p. 457.)

Until 1914,<sup>1</sup> half normal potassium bichromate solution was used as the standard for measuring the color obtained in the reaction of creatinine with picric acid and alkali hydroxide. "That the bichromate has been serviceable for the purpose is evidenced by the fact that no other standard has ever been proposed. Potassium bichromate is, however, by no means ideal as a general standard of measure for the color comparisons involved in creatinine determinations. For determinations in ordinary urines containing 7 to 15 mg. of creatinine in a volume of 5 to 15 cc. it may be regarded as satisfactory. For more dilute urines (or creatinine solutions) obtainable in unlimited quantities, a fair degree of accuracy can be obtained with the bichromate standard by using the proportions of picric acid and alkali recommended by Shaffer."<sup>2</sup>

"The use of the rigid bichromate standard imposes distinct and wholly unnecessary limitations on the applications of a remarkably flexible analytical method, and now that pure creatinine compounds can be prepared from urine with very little work, and pure creatinine is not costly, it seems a mistake to continue the use of the bichromate except perhaps for purely routine determinations in the course of ordinary urine analysis."<sup>3</sup>

<sup>1</sup> O. Folin, *J. Biol. Chem.*, **17**, 469 (1914).

<sup>2</sup> *J. Biol. Chem.*, **18**, 527 (1914).

<sup>3</sup> O. Folin, *J. Biol. Chem.*, **17**, 469 (1914).

**Reagents.**

1. Picric acid. Prepare a saturated solution. For the preparation of picric acid, see page 457.

2. Sodium hydroxide, 10 per cent.

3. Standard creatinine solution. Dissolve 1 gram of pure creatinine (or, better, 1.602 grams of creatinine zinc chloride) in sufficient 0.1 N hydrochloric acid to make a liter, and mix thoroughly. One cubic centimeter contains 1 mg. of creatinine.

For the preparation of pure creatinine, see Folin, J. Biol. Chem., **17**, 163 (1914) and Benedict, *ibid.*, **18**, 182 (1914). See also Edgar and Hinegardner, *ibid.*, **56**, 881 (1923). For Edgar's method of preparing pure creatinine zinc chloride, see page 459.

**Procedure (Folin).**<sup>4</sup>—One cubic centimeter of the standard creatinine solution is measured into a 100 cc. volumetric flask and 1 cc. of the urine into another; 20 cc. of saturated picric acid solution (measured with a cylinder) are added to each and then 1.5 cc. of 10 per cent sodium hydroxide solution. At the end of 10 minutes the flasks are filled up to the mark with water and the color of the unknown is determined.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \text{mg. of creatinine in the amount of urine used.}$$

If the urine shows less than two-thirds or more than one and one-half times the creatinine of the standard the determination should be repeated with more or with less urine.

**Note.**—In case it is desirable to use 5 or 10 cc. of urine (or 5 or 10 mg. of creatinine) the proportions of picric acid and alkali which will give stable colored solutions should be determined.

**DETERMINATION OF CREATINE**

When creatine is boiled with acid it is converted into creatinine by dehydration. By determining the creatinine content before and after the acid treatment, the amount of creatine originally present can be calculated. One gram of creatinine  $\approx$  1.159 grams of creatine.

<sup>4</sup> J. Biol. Chem., **17**, 469 (1914).

METHOD A: THE FOLIN-BENEDICT METHOD<sup>5</sup>**Reagents.**

1. Hydrochloric acid, 1 N.
2. Picric acid. Use a saturated solution. (For a method of preparing pure picric acid, see p. 457.)
3. Sodium hydroxide, 10 per cent.
4. Rochelle salt.
5. Lead. Use powdered or granulated metallic lead.
6. Standard creatinine solution. (See p. 459.)

**Procedure.**—Measure out a volume of urine which contains between 7 and 12 mg. of total creatinine and transfer it to a small flask or beaker. Add 10 to 20 cc. of 1 N hydrochloric acid and a pinch or two of powdered or granulated lead. Boil the mixture over a free flame as slowly or as rapidly as may be desired, until very nearly down to dryness, and then continue heating to dryness either on a water-bath, or by simply holding the vessel in the hand and heating carefully for a moment or two. Let the residue stand on a water-bath for a few minutes until most of the excess of hydrochloric acid has been expelled, after which it is dissolved in about 10 cc. of hot water and the solution rinsed quantitatively through a plug of cotton or glass wool (to remove all metallic lead) into a 500 cc. volumetric flask. Add 20 to 25 cc. of saturated picric acid and 7 or 8 cc. of 10 per cent sodium hydroxide solution, which contains about 5 per cent of Rochelle salt. (See Note 1.) Fill the flask to the mark with water, mix thoroughly, and at the end of 5 minutes compare against a standard.

**Calculation.**—Calculate the creatinine of the solution in the same way as given on page 519. From this value subtract the value for the creatinine content of the urine before dehydration. The difference is the creatine content of the original urine in terms of creatinine.

**Notes.**

1. The Rochelle salt is added to prevent any formation of turbidity, which otherwise may occur, owing to the presence of traces of dissolved lead. The tartrate has no effect whatever upon creatinine readings.

2. The above method "has been tested with pure creatine solu-

<sup>5</sup> Benedict, J. Biol. Chem., 18, 191 (1914).

tions of widely varying concentration (from 1 mg. to 1 gram in 20 cc.), with normal and creatine-containing urines (human and dog), with urines to which known amounts of creatine have been added, and with beef and muscle extracts. The results have been invariably within the limits of accuracy of the colorimeter. . . . It is not, however, applicable to urines containing glucose."<sup>6</sup>

For diabetic urines use the following procedure of Folin:<sup>7</sup> Mix 10 cc. of urine and 5 cc. of 1 N hydrochloric acid and heat the solution on a water-bath for 3 hours. Then dilute to 50 cc., and mix. Neutralize 25 cc. of the diluted solution and determine the "total creatinine" as in the case of creatine alone.

The slight darkening of the urine-hydrochloric acid mixture upon heating does not interfere with the analysis, since the solution is subsequently diluted.

#### METHOD B: MICROCHEMICAL METHOD OF FOLIN<sup>8</sup>

Enough urine to give 0.7 to 1.5 mg. of creatinine is measured into a weighed Erlenmeyer Pyrex flask (capacity 200 cc.). To this are added 20 cc. of saturated picric acid solution, about 130 cc. of water, and a few very small pebbles to promote even boiling. The mixture is gently boiled (preferably over a micro-burner) for about one hour. At the end of this time, increase the heat and boil down the solution to a little less than 20 cc. Weigh, and add enough water to make the total solution equal to 20 to 25 grams. Cool the solution in running water, add 1.5 cc. of 10 per cent sodium hydroxide solution and determine the "total creatinine" as in the pre-formed creatinine using 1 mg. of creatinine as standard.

#### Notes.

1. The above procedure has been found to give quantitative results even in the presence of as much as 25 mg. of urea nitrogen, and 50 mg. of glucose, cane sugar, lactose, or levulose.

2. By heating the urine-acid mixture in an autoclave at a temperature of 117 to 120° C., the analysis may be considerably shortened.

<sup>6</sup> Benedict, J. Biol. Chem., **18**, 193 (1914).

<sup>7</sup> Z. physiol. Chem., **41**, 222 (1904).

<sup>8</sup> J. Biol. Chem., **17**, 472 (1914).



### DETERMINATION OF URIC ACID BY THE BENEDICT AND FRANKE DIRECT COLORIMETRIC METHOD<sup>9</sup>

This method is based upon the blue color obtained when diluted urine is treated directly with an arseno-phosphotungstic acid reagent and sodium cyanide.

#### Reagents.

1. Sodium cyanide, 5 per cent. (Poisonous!) Prepare fresh about every 2 months.

2. Arseno-phosphotungstic acid reagent. Dissolve 100 grams of pure sodium tungstate in about 600 cc. of water, add 50 grams of pure arsenic pentoxide, 25 cc. of 85 per cent phosphoric acid, 20 cc. of concentrated hydrochloric acid, boil the mixture for about 20 minutes, cool, dilute to a liter, and mix. The reagent apparently keeps indefinitely.

3. Standard uric acid solution. Prepare a standard containing 0.02 mg. of uric acid per cubic centimeter. (See p. 462.)

**Procedure.**—Dilute the urine so that 10 cc. contain between 0.15 and 0.30 mg. of uric acid. A dilution of 1 to 20 is generally satisfactory. Measure 10 cc. of the diluted urine into a 50 cc. volumetric flask, add from a burette 5 cc. of 5 per cent sodium cyanide solution and then 1 cc. of the arseno-phosphotungstic acid reagent. Mix by gentle shaking, let stand for 5 minutes, dilute to the mark with distilled water, and mix thoroughly. Compare the resulting blue solution in a colorimeter with a standard prepared simultaneously by treating 10 cc. of the standard uric acid solution (0.2 mg. of uric acid) in a 50 cc. volumetric flask in the same way as directed for the unknown.

#### Calculation.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.2 = \text{mg. of uric acid in 10 cc. of the diluted urine.}$$

### DETERMINATION OF URIC ACID BY THE FOLIN AND WU COLORIMETRIC METHOD<sup>10</sup>

This method is based upon the blue color obtained when phosphotungstic acid and alkali are added to a solution of uric acid. A similar

<sup>9</sup> J. Biol. Chem., **52**, 387 (1922).

<sup>10</sup> Folin and Wu, J. Biol. Chem., **38**, 459 (1919); Folin, Laboratory Manual of Biological Chemistry, p. 141, D. Appleton and Co., New York, 1926.

color may be produced by certain other substances in urine, and hence it may be necessary to separate the uric acid from such substances. This is accomplished by adding silver lactate which precipitates silver urate. The latter is then dissolved and treated with the uric acid reagent.

#### Reagents.

1. Sodium carbonate. Use a saturated solution.
2. Sodium cyanide, 5 per cent. (Poisonous!)
3. Sodium sulfite, 10 per cent. Keep in a small, tightly stoppered bottle.
4. Silver lactate reagent. Prepare a solution containing 5 per cent of silver lactate and 5 per cent of lactic acid.
5. Phosphotungstic acid reagent of Folin and Denis. Boil 100 grams of sodium tungstate with 80 cc. of 85 per cent phosphoric acid and about 700 cc. of water for at least 2 hours. If the resulting solution is very dark, add a few drops of bromine and boil for about 15 minutes to remove the excess bromine. Cool, dilute to a liter, and mix.
6. Standard uric acid solution. Dissolve 1 gram of uric acid in about 150 cc. of 0.4 per cent lithium carbonate solution, dilute to 500 cc. in a volumetric flask, and mix thoroughly. Transfer 50 cc. (corresponding to 100 mg. of uric acid) to each of a series of volumetric liter flasks. Add to each flask about 300 cc. of water and 500 cc. of clear (filtered) 20 per cent sodium sulfite solution, mix, dilute to volume, and mix thoroughly. One cubic centimeter of the diluted solution contains 0.1 mg. of uric acid.

Fill a series of 200 cc. bottles with the standard solution, and stopper tightly. The use of a series of small bottles is to reduce the absorption of oxygen from the air.

**Procedure.**—Transfer from 1 to 3 cc. of urine to a centrifuge tube and add enough water to bring the volume up to about 6 cc. Add 5 cc. of the silver lactate reagent, stir with a very fine glass rod, rinse off the rod with a few drops of water, and centrifuge. If enough silver lactate has been added, the precipitate settles very quickly. Add a drop of the silver lactate reagent in order to be sure that an excess is present. If a precipitate is formed, add 2 cc. more of the silver lactate reagent and again centrifuge. The first 5 cc. of the silver lactate is almost always sufficient, but it is not safe to omit the test. Pour off the clear

supernatant liquid as nearly completely as possible, being very careful not to remove any of the precipitate.

Add to the precipitate in the tube 4 cc. of 5 per cent sodium cyanide solution. (Poisonous!) The cyanide solution should be added from a burette. Stir until a clear solution is obtained and pour into a 100 cc. volumetric flask, rinsing the tube and stirring rod with 15 to 25 cc. of water. Add 5 cc. of 10 per cent sodium sulfite solution (to balance the sulfite in the standard uric acid solution). Dilute to a volume of about 50 cc.

Transfer to another 100 cc. flask 5 cc. of the standard uric acid sulfite solution (containing 0.5 mg. of uric acid), add 4 cc. of the cyanide solution, and dilute to about 50 cc. Then add 20 cc. of saturated sodium carbonate solution to each flask, mix, and finally add *with shaking* 2 cc. of the phosphotungstic acid reagent. Let stand for 3 to 5 minutes, fill to the mark, mix thoroughly, let settle, pour off the clear supernatant liquids into colorimeter cups and make the comparison in the usual way, never omitting to read, first, the standard against itself. Artificial light (with "daylite" glass) is better than daylight for this color comparison.

#### *Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.5 = \text{mg. of uric acid in the amount of urine used.}$$

#### **Notes.**

1. Precaution! Pour the discarded blue solutions directly into the drain pipes on account of the cyanide.
2. Folin<sup>11</sup> recommends the use of formalin instead of the sulfite in the preparation of the standard uric acid solution.

#### **DETERMINATION OF SUGAR BY THE METHOD OF SUMNER<sup>12</sup>**

Dinitrosalicylic acid reagent is added to urine and the solution heated. The sugar reduces the dinitrosalicylic acid and the resulting colored solution is compared against a standard. The reagent is entirely satisfactory for the determination of sugar both in normal and in diabetic urine. The intense color obtained with normal urine does not change in value for one-half hour.

<sup>11</sup> J. Biol. Chem., **54**, 159 (1922).

<sup>12</sup> J. Biol. Chem., **65**, 393 (1925).

**Reagent.**—To 10 grams of crystallized phenol add 22 cc. of 10 per cent sodium hydroxide. Dissolve in a little water and dilute to a volume of 100 cc. Weigh out 6.9 grams of sodium bisulfite and add to this 69 cc. of alkaline phenol solution. Now add a solution containing 300 cc. of 4.5 per cent sodium hydroxide, 255 grams of Rochelle salt ( $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ), and 880 cc. of 1 per cent dinitrosalicylic acid solution. Mix and keep tightly stoppered in well-filled bottles.

**Procedure.**—Pipette into a Folin-Wu sugar tube 1 cc. of urine (diluted if necessary) and 3 cc. of the reagent. Mix and heat 5 minutes in boiling water. Cool 3 minutes in running water, dilute to 25 cc. volume, mix, and compare in a colorimeter with a standard prepared with 1, 0.5, or 0.25 mg. of glucose, according to the concentration of sugar in the urine.

#### DETERMINATION OF SUGAR BY THE METHOD OF BENEDICT AND OSTERBERG<sup>13</sup>

This method is based upon the red to brown color obtained by heating glucose solutions with picric acid and alkali.

##### Reagents.

1. Picric acid, 0.6 per cent. This solution is best prepared from dry picric acid.
2. Sodium hydroxide, 5 per cent.
3. Acetone, 50 per cent. Prepare fresh every day or two by diluting pure acetone with an equal volume of water.
4. Purified bone-black. Treat 250 grams of commercial bone-black with 1.5 liters of 1 : 4 hydrochloric acid and boil the mixture for about 30 minutes. Filter on a large Büchner funnel and wash the bone-black with hot water until the washings are neutral to litmus. The bone-black is then dried and powdered. Test the product before using by shaking 15 cc. of a glucose solution (containing 0.5 mg. of glucose per cubic centimeter) with 1 gram of the bone-black and determining the sugar in the filtrate. There should be no detectable absorption of the sugar by the bone-black. The highly absorbent animal charcoals now on the market should not be used for this purpose.
5. Standard glucose solution. Prepare a solution containing 1 mg. of pure glucose per 3 cc. The solution will keep indefinitely if preserved with a little toluene.

<sup>13</sup> J. Biol. Chem., **48**, 51 (1921).

**Procedure.**—Treat 15 cc. of urine (diluted to a specific gravity of not over 1.030) with about 1 gram of purified bone-black. (Smaller amounts of urine and bone-black may be used.) Shake the mixture vigorously occasionally for a period of 5 to 10 minutes and then filter through a small, dry filter into a dry flask or beaker. From 1 to 3 cc. of the filtrate (containing about 1 mg. of sugar) are transferred to a test tube graduated at 25 cc. If less than 3 cc. of filtrate is used, dilute to exactly 3 cc. with distilled water. Add exactly 1 cc. of 0.6 per cent picric acid solution and 0.5 cc. of 5 per cent sodium hydroxide solution. Next add 5 drops of 50 per cent acetone solution, taking care that the drops fall directly into the solution and not on the wall of the tube. Mix the contents of the tube, insert a cotton plug, and heat in boiling water for 12 to 15 minutes. The standard solution should be prepared simultaneously with the unknown. This is done by treating 1 mg. of sugar in 3 cc. of solution exactly as described for the unknown.

After heating, both tubes are removed from the boiling water, cooled to room temperature, made up to 25 cc. with water, mixed, and compared.

*Calculation.*

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \text{mg. of sugar in the volume of diluted urine used.}$$

If the original diluted urine was diluted further, a correction for this must of course be made.

**Note.**—In connection with the use of the above method, Benedict and Osterberg<sup>14</sup> call attention to the following points: "The quantity of the picric acid solution used must be measured with exactness, just as are the unknown and standard sugar solutions. Slight variations in the alkali are not so important. Adding the same number of drops (about 10) to each of the tubes from the same pipette is sufficient. The acetone solution should be added last, and the tubes placed in the water-bath within about a minute afterwards. The diluted acetone undergoes some peculiar change on standing which makes old solutions yield somewhat irregular results. It is therefore best to prepare the acetone solution fresh every day or two.

"Each solution should be so added that it falls into the bottom of the tube, and does not hit the sides. Standard and unknown must correspond in sugar content within reasonable limits. For a 1 mg. stand-

<sup>14</sup> J. Biol. Chem., 48, 55 (1921).

ard, satisfactory results can be obtained for an unknown solution containing between 0.75 and 1.75 mg. of sugar. With wider variations between unknown and standard, results are not so good, particularly when the quantity of sugar is low. If less than 0.7 mg. of sugar is present in the unknown, it is better to have a standard solution containing 0.5 mg. of sugar in 3 cc., and to dilute both unknown and standard to 12.5 instead of to 25 cc."

#### DETERMINATION OF PROTEINS BY THE METHOD OF FOLIN AND DENIS<sup>15</sup>

This is a turbidimetric method. The albumin of the urine is precipitated with sulfosalicylic acid and the resulting turbidity compared with that of a standard protein solution.

##### Reagents.

1. Sulfosalicylic acid, 25 per cent.
2. Standard protein solution. About 30 cc. of *fresh* blood serum (free from hemoglobin) are diluted with a 15 per cent solution of pure sodium chloride to about 1500 cc. The solution is thoroughly mixed and then filtered. By means of nitrogen determinations the protein content of the filtrate is determined ( $\text{protein} = N \times 6.25$ ), and on the basis of the figure obtained the solution is diluted with 15 per cent sodium chloride solution so that it contains 2 mg. of protein per cubic centimeter. It is best to saturate the protein solution with chloroform (20 cc.). The solution will keep for at least several months. As a matter of further precaution, the stock solution may be kept in a refrigerator.

**Procedure.**—To about 75 cc. of water in each of two 100 cc. volumetric flasks, are added 5 cc. of a 25 per cent solution of sulfosalicylic acid. To one flask is then added 5 cc. of the standard protein solution containing 10 mg. of albumin, and to the other is added the albuminous urine 1 cc. at a time (by means of an Ostwald pipette) until the turbidity obtained seems to be reasonably near that of the standard. The two flasks are then filled up to the mark with water, cautiously inverted a few times to secure mixing, and are then ready for the quantitative comparison in the colorimeter tubes. The standard must invariably first be read against itself to secure the adjustment of the colorimeter (and of the eye). The contents of one of the colorimeter

<sup>15</sup> J. Biol. Chem., 18, 273 (1914).

cups is then replaced by the suspension of the unknown, and the turbidity comparison is made exactly as in colorimetric work.

The standard, containing 10 mg. of protein, is read with the unknown set at 20 mm. It must not read less than 10 nor more than 30 mm.

*Calculation.*—Dividing the reading of the standard by twice the number of cubic centimeters of urine taken gives the albumin in milligrams per cubic centimeter of urine.

#### Notes.

1. The albuminous suspensions must be mixed very gently so as to avoid agglutination.
2. The method is fairly accurate and can be carried out in a few minutes, provided only that a standard albumin solution is available.
3. The method cannot be applied to urines very deeply colored with blood or bile pigments. It is, of course, applicable to various albuminous fluids (not highly pigmented), for example, exudates, transudates, and the cerebrospinal fluid.
4. It must be remembered that different proteins may give very different degrees of turbidity under the same experimental conditions.<sup>16</sup>

#### DETERMINATION OF PHENOLS BY THE METHOD OF FOLIN AND DENIS<sup>17</sup>

This method is based upon the deep blue color obtained when phenol solutions are treated with phosphotungstic-phosphomolybdic acid and alkali. Since traces of protein, which may be present in the urine, and uric acid also give a blue color with the reagent, they must be removed prior to the phenol determination. Their removal is accomplished by precipitation with silver lactate, in dilute lactic acid solution, and colloidal hydrated ferric oxide.

#### Reagents.

1. Hydrochloric acid, sp. gr. 1.19.
2. Sodium chloride. Prepare a saturated solution of sodium chloride, containing 10 cc. of concentrated hydrochloric acid per liter.

<sup>16</sup> Marshall and Banks, Proc. Am. Phil. Soc., **54**, 176 (1915).

<sup>17</sup> O. Folin and W. Denis, J. Biol. Chem., **22**, 305 (1915).

3. Sodium carbonate. Use a saturated solution.
4. Colloidal hydrated ferric oxide solution.
5. Silver lactate. Prepare a 3 per cent solution of silver lactate in 3 per cent lactic acid.
6. Phosphotungstic-phosphomolybdic acid reagent. Mix 100 grams of sodium tungstate, 20 grams of phosphomolybdic acid (or an equivalent amount of molybdic acid), 50 cc. of 85 per cent phosphoric acid, and 75 cc. of water. Boil the solution for 2 hours, cool, dilute to a liter with distilled water and filter if necessary.
7. Standard phenol solution. Prepare a solution of pure phenol in 0.01 N hydrochloric acid. Standardize by an iodometric titration and adjust the concentration so that 10 cc. of the standard contains 1 mg. of phenol.

The preparation is made as follows: Make a phenol solution in 0.1 N hydrochloric acid, which contains a *little more* than 1 mg. of crystallized phenol per cubic centimeter. Transfer 25 cc. of the solution to a 250 cc. flask, add 50 cc. of 0.1 N sodium hydroxide solution, heat to 65° C., add 25 cc. of 0.1 N iodine solution, stopper the flask, and let stand at room temperature for 30 or 40 minutes. Add 5 cc. of concentrated hydrochloric acid and titrate the excess of iodine with 0.1 N sodium thiosulfate solution, using starch solution as an indicator. Do not add the starch solution until the end-point is almost reached. Each cubic centimeter of 0.1 N iodine solution corresponds to 1.567 mg. of phenol. On the basis of the result dilute the phenol solution so that 10 cc. contain 1 mg. of phenol.

To prepare a standard solution for comparison, place 5 cc. of the standard phenol solution (equivalent to 0.5 mg. of phenol) in a 100 cc. volumetric flask, add 10 cc. of the phosphotungstic-phosphomolybdic acid reagent and 25 cc. of saturated sodium carbonate solution, dilute to the mark with water at about 30° C. and mix thoroughly.

**Procedure A: Preliminary Treatment.**—Transfer 10 cc. of ordinary, or 20 cc. of very dilute, urine to a 50 cc. volumetric flask and add acid silver lactate solution (2 to 20 cc.) until no further precipitate forms. Next add a few drops of colloidal ferric oxide solution, shake, dilute to the mark with distilled water, again shake, and filter the contents through a small, dry filter. This precipitation removes quantitatively uric acid and traces of protein, both of which give a blue color with the phenol reagent. The phenols are recovered quantitatively in the filtrate. Transfer 25 cc. of the filtrate to a 50 cc. volumetric flask



and add to it a sufficient quantity of saturated sodium chloride solution, containing 10 cc. of concentrated hydrochloric acid per liter, to precipitate all the silver. The flask is then filled to the mark with distilled water and its contents mixed and filtered through a small, dry filter. The filtrate is used for the determination of free and total phenols.

**Procedure B: "Free" (Non-conjugated) Phenols.**—Place 20 cc. of the above filtrate in a 50 cc. volumetric flask and add 5 cc. of the phosphotungstic-phosphomolybdic acid reagent and 15 cc. of saturated sodium carbonate solution. Dilute to volume with lukewarm water (30–35° C.), mix thoroughly, and after 20 minutes compare in a colorimeter against a standard phenol solution prepared as directed above.

**Procedure C: Total (Free and Conjugated) Phenols.**—Transfer to a large test tube 20 cc. of the filtrate obtained in A, add 10 drops of hydrochloric acid, sp. gr. 1.19, cover the tube with a small funnel, heat the mixture rapidly to boiling over a free flame, and then place the tube in a beaker (tall form) of boiling water for 10 minutes. The tube is then removed, cooled, and its contents transferred to a 100 cc. volumetric flask. Add 10 cc. of phosphotungstic-phosphomolybdic acid reagent, 25 cc. of saturated sodium carbonate solution, make up to volume with distilled water, mix thoroughly, and after 20 minutes compare in a colorimeter against a standard phenol solution prepared as directed above.

*Calculation.*—In making the calculation it must be remembered that aliquot parts are employed, one-fifth of the original sample being finally used for each determination. Furthermore, the final dilution in the case of "free" phenols is half that of the standard, while in the case of total phenols it is the same as that of the standard.

Hence,

$$\frac{\text{Reading of standard}}{\text{Reading of unknown} \times 4} = \text{mg. of free phenols,}$$

and

$$\frac{\text{Reading of standard}}{\text{Reading of unknown} \times 2} = \text{mg. of total phenols,}$$

in 2 cc. or 4 cc. of urine, depending upon whether 10 cc. or 20 cc. of urine were taken for the analysis.

**DETERMINATION OF MANGANESE BY THE METHOD OF McCrackan  
AND PASSAMANECK<sup>18</sup>**

The manganese is oxidized to permanganic acid by ammonium persulfate and silver nitrate in the presence of nitric acid and the resulting pink solution is then matched against a standard permanganate solution.

**Reagents.**

1. Nitric acid, sp. gr. 1.42.
2. Sulfuric acid, sp. gr. 1.84.
3. Silver nitrate, 0.1 N.
4. Ammonium persulfate, 50 per cent.

**Procedure.**—To 100 cc. of urine in a Kjeldahl flask, 20 cc. of concentrated nitric acid are added. If the urine contains less than 1 mg. of manganese per liter of urine, larger amounts in the same proportion are used. This is followed by evaporation to a paste on a sand-bath; after cooling, 5 cc. of concentrated sulfuric acid are added. The preparation is then heated at high temperature until about a third of the acid is driven off as heavy white fumes. With urine high in phosphates, or when a large quantity of urine is used for the analysis, the amount of sulfuric acid may have to be increased. After cooling, 5 cc. of concentrated nitric acid is added, followed by heating until brown fumes disappear. If oxidation does not seem to be complete, this step is repeated again and again until there is no doubt, more sulfuric acid being added if necessary. After cooling, transfer is made to a 100 cc. volumetric flask by means of about 75 cc. of distilled water. Then 5 cc. of concentrated nitric acid, 1 cc. of tenth normal silver nitrate, and 1 cc. of 50 per cent ammonium persulfate are added and diluted to the mark. One or more standards are prepared with similar amounts of reagents from manganous sulfate or manganous nitrate, or potassium permanganate in 100 cc. flasks, and both the known and the unknown are heated at the same time in the water-bath until the formation of permanganic acid is complete. If the depth of color in the unknown is deep enough, comparison is made in the colorimeter, or in Nessler tubes if it is too faint. When manganese is found a "blank" test should be made on the reagents as a control.

<sup>18</sup> Arch. Path. Lab. Med., 1, 585 (1926).

**Notes.**

1. In case the acidity is already quite high because more than the recommended amount of sulfuric acid has been used, less nitric acid may be used before adding the persulfate, or its use may be omitted. In case much silica from the glassware is present, it may be ignored until after the solution is made up to volume and the color developed. Then it may be removed from a portion by centrifuging, or it may be allowed to settle by gravity.

2. McCrackan and Passamaneck made a series of about forty analyses by the above procedure, and then one of them prepared four unknowns which were then analyzed by the other. The results are given in Table XLIII.

TABLE XLIII

Urine Used for Analysis, Cubic Centimeters	Manganese Present, Milli- grams per Liter	Manganese Found, Milli- grams per Liter
100	2.04	2.18
100	0.96	0.93
100	2.00	2.08
200	0.22	0.21

3. While it would seem possible to find one part of manganese in several billions of distilled water by condensing a large volume to a small one before applying the test, there is a limit to this procedure with urine because of the difficulty in getting rid of the phosphates. It is difficult to make use of more than 2 liters in any analysis, and it is desirable to work with smaller quantities. It is obvious that reducing substances coming in contact with the permanganic acid will decolorize it and cause negative or low results. The metallic parts of the colorimeter cups must not be overlooked in this connection. On the other hand, the test is so sensitive that the clinician cannot be too careful about protecting the specimen of urine from contamination. The dust from the air of a manganese grinding plant or that from a worker's clothes might easily cause analyses on specimens of normal urine to be positive, or those on pathologic specimens to run much higher than

they should. Urine contaminated by feces, in experimental work with animals, may cause wrong conclusions.

The amount of manganese in one normal urine has been found to be less than 1 part in 50,000,000.

#### DETERMINATION OF HYDROGEN ION

For a discussion of the principles of the colorimetric determination of hydrogen-ion concentration, see Chapter XIX.

##### Reagents.<sup>19</sup>

1. Bromocresol green ( $pH$  4.0 to 5.6), bromocresol purple ( $pH$  5.4 to 7.0), phenol red ( $pH$  6.6 to 8.2). Use aqueous solutions of 0.04 per cent strength. Add 0.5 cc. of these indicator solutions to 10 cc. portions of the standard buffer solutions.

2. Standard buffer solutions. Prepare according to Clark: *The Determination of Hydrogen Ions*, 2d ed., Williams & Wilkins Co., Baltimore, Md., 1922.

**Procedure.**—Collect the urine and keep it under a mineral oil. Place 8 cc. of redistilled and recently boiled distilled water in a test tube the same size as employed for the color standards. Add 0.5 cc. of the standard indicator solution and cover with a layer of mineral oil. Then introduce under the oil 2 cc. of the urine. Gently mix and match against the indicator standards in a comparator block. Run a control tube containing the urine diluted 1 to 5. The arrangement of the tubes in the comparator block is shown on page 211.

The observed  $pH$  value obtained at room temperature may be approximately corrected to give the actual  $pH$  at 38° C., by subtracting 0.2  $pH$ . This correction is not a constant factor. (See Note 3, p. 493.)

##### Notes.

1. The  $pH$  values of normal urines range from 5.5 to 8.0 with a mean value of almost exactly 6.0. In the case of cardio-renal disorders the mean  $pH$  is 5.3, and for vegetarians about 6.6.

2. Myers' multiple wedge colorimeter (bi-colorimeter)<sup>20</sup> is convenient for  $pH$  work.

<sup>19</sup> Indicators and standard buffer salts, both dry and in prepared solutions, may be obtained from the LaMotte Chemical Products Co., Baltimore, Md.; or Hynson, Westcott and Dunning, Baltimore, Md.

<sup>20</sup> *J. Biol. Chem.*, **54**, 675 (1922). This colorimeter is manufactured by E. Leitz, Inc., New York.

**DETERMINATION OF LEAD**

See page 258.

**DETERMINATION OF ZINC**

See page 397.

**DETERMINATION OF NICKEL**

See page 298.

**DETERMINATION OF CALCIUM**

See pages, 139, 143, and 146.

**DETERMINATION OF MAGNESIUM**

See page 264.

**DETERMINATION OF PHOSPHATE**

See pages 346, 348 and 353.

**DETERMINATION OF CHLORIDE**

See page 159.

**DETERMINATION OF SILICON**

See page 366.

For the nephelometric determination of a number of substances in biological material, see Volume II.

## PART V

### BIBLIOGRAPHY

#### BIBLIOGRAPHY ON COLORIMETRY ARRANGED ALPHABETICALLY BY SUBJECT AND CHRONOLOGICALLY UNDER EACH SUBJECT

THERE are two outstanding characteristics that a reference work should embody, namely, accuracy and completeness. But there is a third feature of almost equal importance: the references should include something of the subject-matter contained in the original articles. Frequently the title of a paper does not indicate important parts of the text. Hence, if a comprehensive survey of a subject is to be made it is necessary not only to examine carefully the indexes of various journals and books, but also to make a page-by-page search of the contents of each article and in turn search the references listed in the various articles. Fortunately, there are a number of abstract journals which greatly lighten the task, particularly in the case of journals having a very limited distribution, or the hundreds of journals dealing with "border-line" subjects or even so-called different branches or fields of knowledge. But these "abstract references" should serve only as a starting point, and in every case, if reasonably possible, the *original* source should be carefully examined.

It has been the aim of the author to make the following bibliography an accurate and fairly complete survey of the literature on colorimetric quantitative analysis. No doubt there are a number of articles that have escaped his attention, and he hopes that the users of the bibliography will call his attention to omissions in order that they may be included in a future edition. Also, notice of any errors will be greatly appreciated.

It should be pointed out that no attempt has been made to include the hundreds of *qualitative* colorimetric tests that are recorded in the literature. A few have been added because of historical interest or

because they seem to offer a promise of being developed into quantitative methods. To have included references to qualitative methods would have considerably increased the size of the bibliography without a proportionate increase in its usefulness.

The official abbreviations of Chemical Abstracts have been used. See "List of Periodicals Abstracted by Chemical Abstracts with Key to Library Files," Chemical Abstracts, **20**, No. 20, Part 2 (1926).<sup>1</sup> In a few instances the references are not listed in Chemical Abstracts but it is believed that the abbreviations adopted will be readily recognized. In general, the first reference given after an author's name is the journal in which the article originally appeared; succeeding references are abstracts of the article. Although many abstract references are to be found in the bibliography, no attempt has been made to give one or more abstract reference on each article.

Many of the references contain a brief abstract, while others have been recorded with only the subject heading. It must not be assumed, however, that the relative importance of an article is indicated by the length of its abstract. Indeed, many excellent papers have been recorded with only their titles or subject headings.

The author desires to take this opportunity to acknowledge his appreciation of the very great assistance rendered him by Chemical Abstracts' "List of Periodicals." It has been a frequent source of aid.

Finally, the author wishes to express his appreciation to the following libraries whose files of periodicals have made this bibliography possible:

Army Medical Library, Washington, D. C.  
Department of Agriculture, Washington, D. C.  
Chemists' Club, New York City.  
Geological Survey, Washington, D. C.  
John Crerar Library, Chicago, Ill.  
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<sup>1</sup> Compiled by the Research Information Service of the National Research Council under the supervision of C. J. West, director.

## BIBLIOGRAPHY

### Acetanilide.

BARTHÉLEMY, H., Caoutchouc and gutta-percha **9**, 6638 (1912); J. Soc. Chem. Ind. **31**, 1026 (1912). Determination of acetanilide in celluloid.

### Acetic Acid.

SCHALL, H., Münch. med. Wochschr. **66**, 812 (1919); Z. anal. Chem. **61**, 80 (1923).

### Acetoacetic Acid.

MARTZ, F., Union Pharmaceut., May 15, 1898; Rev. chim. anal. appl. **6**, [10], 165-166; Analyst **23**, 235 (1898). Determines acetoacetic acid in diabetic urine.

FOLIN, O. and DENIS, W., J. Biol. Chem. **18**, 263 (1914). Turbidity methods for the determination of acetone, acetoacetic acid and  $\beta$ -oxybutyric acid in urine.

SCHALL, H., Münch. med. Wochschr. **66**, 812 (1919); J. Chem. Soc. **118**, ii, 63 (1920); C. A. **14**, 1555 (1920); Chem. Zentr. **1919**, iv, 562. Estimation of acetone and acetoacetic acid with Autenrieth's colorimeter.

LENK, E., Münch. med. Wochschr. **66**, 1119 (1919); Chem. Zentr. **1919**, iv, 995. Says Schall, *ibid.* **66**, 812 (1919), simply adopted L's method and substituted a colorimeter for the measuring cylinder.

### Acetone.

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Says Schall, *ibid.* **66**, 812 (1919), simply adopted L's method and substituted a colorimeter for the measuring cylinder.

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- SITSEN, M. H. P., *Pharm. Weekblad* **62**, 622 (1925). Colorimetric determination of acetone in urine. "To 50 cc. of urine add 10 cc. basic Pb acetate soln. Add 10 cc. of 10 per cent  $\text{Na}_2\text{HPO}_4$  to 30 cc. of the filtrate, dil. to 50 cc. and filter through a triple filter. The filtrate is colorless and corresponds to one-half the vol. of urine. To 5 cc. of this decolorized urine add 2 cc. of 5 per cent Na nitroprusside soln. and 5 cc. 4 N NaOH and mix, then add 10 cc. 30 per cent AcOH and dil. to 50 cc. By means of a colorimeter compare the red color with that of 0.0005 per cent McAc soln. treated simultaneously in the same manner. The method is not applicable to urines contg. phenolphthalein or istizin (synthetic purgatives)." A. W. Dox, C. A. **19**, 2507 (1925).
- BEHRE, J. A. and BENEDICT, S. R., *J. Biol. Chem.* **70**, 487 (1926). Acetone bodies in blood and urine.

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- ILOSVAY DE NAGY ILOSVA, L., *Ber.* **32**, 2697 (1899). On the preparation of ammoniacal cuprous salt solution by hydroxylamine for testing acetylene. Prepares cuprous salts by reduction of cupric salts with hydroxylamine.
- KÜSPERT, F., *Z. anorg. Chem.* **34**, 453 (1903). Note on colloidal copper acetylene. Observes that  $\text{CuCl}_2$  gives a red colloidal solution when added to an aq. acetylene solution. Apparently no attempt to use the reaction analytically.
- WEAVER, E. R., *J. Am. Chem. Soc.* **38**, 352 (1916) and *Bur. Standards, Sci. Paper No. 267*, 28 (1916); *J. Soc. Chem. Ind.* **35**, 347 (1916); *Z. anal. Chem.* **58**, 292 (1919); *J. Franklin Inst.* **181**, 408 (1916). Uses ammoniacal cuprous chloride solution containing gelatin. A red colloidal solution formed, stabilized by the gelatin.
- SCHULZE, A., *Z. angew. Chem.* **29**, 341 (1916); *J. Soc. Chem. Ind.* **35**, 1054 (1916); *C. A.* **11**, 1114 (1917). Uses ammoniacal cuprous chloride. A red colloidal solution is formed.

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- FRÖWEIN, B., *Biochem. Z.* **134**, 559 (1923); *C. A.* **17**, 1650 (1923).
- FRIEND, H., *J. Biol. Chem.* **57**, 497 (1923); *J. Chem. Soc.* **126**, ii, 75 (1924).
- A quantitative color reaction given by adrenaline and urine. Treats successively with sulphanilic acid,  $\text{HNO}_3$ , and ammonia. Red coloration obtained.

#### Albumin (See also Protein).

- JOHNSON, G., *Brit. Med. J.* **1883**, 504. Uses picric acid and  $\text{KOH}$ .
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- RIEGLER, E., *Z. anal. Chem.* **53**, 242 (1914); *J. Chem. Soc.* **103**, ii, 395 (1914); *J. Soc. Chem. Ind.* **33**, 442 (1914). Method based upon fact that an alkaline solution of albumin dissolves copper hydroxide, giving a violet colored solution whose intensity is proportional to the amount of albumin present.
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- ROCQUES, X., Ann. chim. anal. **10**, 103 (1905). Compare Compt. rend. **140**, 511; J. Chem. Soc. **88**, ii, 359 (1905). Colorimetric estimation of higher alcohols in brandies.
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- LYONS, A. B., J. Am. Pharm. Assocn. **11**, 12 (1922). Detection of wood spirit in alcoholic beverages. Uses dried egg albumin. Procedure only qualitative. For a quantitative procedure, see *ibid.* **11**, 682.
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- sensitive to 1 part formaldehyde in 1000 parts of solution. A purple ring is obtained. 4. Phenylhydrazine hydrochloride seems to be the best reagent. Will detect 1 part formaldehyde in 250,000 parts of water. A green tinge is obtained. 5. Rimini uses phenylhydrazine hydrochloride with Na nitroprusside and concentrated NaOH. Will detect 1 part formaldehyde in 1,000,000 parts water. A blue coloration is obtained. The blue changes quickly to green, yellow, light brown, and red.
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- ROSENHEIM, O., Analyst **32**, 106 (1907); J. Chem. Soc. **92**, ii, 512 (1907). The Chemistry of Hehner's test for formaldehyde in milk. Action depends on presence of the tryptophan group.
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- SHREWSBURY, H. S. and KNAPP, A. W., Analyst **34**, 12 (1909); J. Chem. Soc. **96**, 192 (1909). Detection and estimation of formaldehyde in milk. Use concentrated HCl containing 0.1 per cent  $\text{HNO}_3$ .
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- FELLENBERG, T. VON, *Chem. Zentr.* **1916**, i, 390; *Mitt. Lebensm. Hyg.* **6**, 254 (1915); *J. Chem. Soc.* **110**, ii, 354 (1916). Colorimetric estimation of cinnamaldehyde in cinnamon. Method depends on the coloration which develops when the aldehyde is treated with  $\text{H}_2\text{SO}_4$  and isobutyl alcohol.
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- JOSEPHSON, K., *Ber.* **56** (B), 1771 (1923); *J. Chem. Soc.* **124**, ii, 665 (1923). Schiff's rosaniline-sulphurous acid reaction for aldehydes. Trustworthy only when the solution has a certain acidity.
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#### Alkaloid.

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#### Allantoin.

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#### Aluminum.

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- ATAK, F. W., *J. Soc. Chem. Ind.* **34**, 936 (1915); *C. A.* **9**, 3186 (1915); *Z. anal. Chem.* **58**, 363 (1919). A new reagent for the detection and colorimetric

- estimation of aluminum. Uses a 1 per cent solution of alizarin-S. Red color. Alizarin-S is the Na salt of alizarinmonosulphonic acid. Glycerol added to test solution to prevent precipitation.  $\text{Al}[\text{C}_{11}\text{H}_6\text{O}_2(\text{OH})_2\cdot\text{SO}_3]_3$  seems to be formed rather than a lake.
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- BALDWIN, F. O., *J. Am. Water Works Assoc.* **12**, 439 (1924); *J. Soc. Chem. Ind.* **44**, B 113 (1925). Colorimetric determination of total alumina in water. Uses alizarin red S (Na alizarinmonosulfonate).
- SCOTT, W. W., *Standard Methods of Chemical Analysis*, 4 ed. p. 14, D. Van Nostrand Co., New York, 1925. Uses alizarin S. Atack's method.
- HAMMETT, L. P. and SOTTERY, C. T., *J. Am. Chem. Soc.* **47**, 142 (1925). A new reagent for aluminum. The dye aurin tricarboxylic acid forms with Al salts a bright red lake which may be used as a qualitative test for Al ion. Interference by Cr is prevented by an unusual property of the Al lake, which when once formed in an HAc-acetate buffer is not decomposed in any reasonable time when the solution is made alkaline with  $\text{NH}_4\text{OH}$ , although it does not form in alkaline solution. The Cr lake, which resembles the Al compound in appearance, forms in an acetate solution, but is decolorized upon the addition of  $\text{NH}_4\text{OH}$ . Under the conditions of the test (1) silicic acid and salts of Bi, Pb, Sb,  $\text{Sn}^{+++}$ ,  $\text{Hg}^{+}$ , Ti give white precipitates; (2) salts of Cd, Zn, Mn, Co, and Ni give no precipitates; (3) Cr, alkaline earths, and phosphate do not interfere if  $\text{NH}_4\text{OH}-(\text{NH}_4)_2\text{CO}_3$  solution is added; (4)  $\text{Fe}^{+++}$  gives a deep violet precipitate in the HAc solution but is converted into a red-dish-brown by  $\text{NH}_4\text{OH}$ . The separation of Fe from Al by  $\text{NaOH}$  or  $\text{Na}_2\text{O}_2$  is sufficient to prevent interference by iron. The delicacy of the test is of the order of  $10^{-6}$  mole of Al. The reagent (the  $\text{NH}_4$  salt of aurin tricarboxylic acid) is now on the market under the trade name of "Aluminon" and may be obtained from the Fales Chemical Co., 74 Cortlandt St., New York City.
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the detection and approximate determination of 0.01-0.1 per cent Al in non-ferrous alloys.

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for only 2 hours. Color and turbidity usually disappear when 2 cc. of the solution are added to 50 cc. of water tested. The difference in color produced by sensitized and nonsensitized solutions is most marked when about 0.005 mg. of  $\text{NH}_3$  is present." W. T. H., C. A. **19**, 2002 (1925).

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#### **Amyl Alcohol.**

ROCQUES, X., *Ann. chim. anal.*, **2**, 221, 222; *Analyst* **22**, 285 (1897).

#### **Aniline.**

TIZARD, H. T., *Proc. Chem. Soc.* Oct. 20, 1910; *Chem. News* **102**, 277 (1910). The hydrolysis of aniline salts measured colorimetrically.

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CHRISTIANSEN, W. G. O., *J. Ind. Eng. Chem.* **11**, 763 (1919). Determination of aniline in dilute aqueous solution.

#### **Anthracene.**

LEWIS, H. F., *Ind. Eng. Chem.* **16**, 1184 (1924); *J. Chem. Soc.* **128**, ii, 74 (1925); C. A. **19**, 452 (1925). Determination of anthracene in anthraquinone. Method based on the fact that when a solution of anthracene in "oleum" is heated, charring takes place, but a similar solution of anthraquinone remains yellow. Dissolve in "10 per cent oleum" heated at  $150^\circ$ , then pour into water, filter, and match color of filtrate against standard solutions of  $\text{K}_2\text{Cr}_2\text{O}_7$  and  $\text{CoCl}_2$ . Accuracy 0.1 per cent up to 7 per cent of anthracene present.

#### **Antimony.**

SCHIDROWITZ, P. and GOLDSBROUGH, H. A., *Analyst* **36**, 101 (1911); C. A. **5**, 2230 (1911); *J. Soc. Chem. Ind.* **30**, 449 (1911). Use gum acacia and  $\text{H}_2\text{S}$ . The acacia is added to ensure colloidal suspension. Can detect 1 part of Sb in 2,000,000 parts of water. Only 70 to 80 per cent of original amount of Sb recovered.

BEAM, W. and FREAK, G. A., *Analyst* **44**, 196 (1919); C. A. **13**, 2323 (1919); *Chem. News* **118**, 236 (1919); *J. Soc. Chem. Ind.* **38**, 515A (1919). Determination as sulfide. An improvement on method of Schidrowitz and Goldsbrough, *Analyst* **36**, 101 (1911), which recovers only 70 to 80 per cent of the original amount of Sb. This method recovers 98 to 103 per cent of the Sb.

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#### Aromatic Hydroxy-Acids.

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#### Arsenic.

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- SNELL, F. D., *Colorimetric Analysis*, p. 64, D. Van Nostrand Co., New York, **1921**. Determination of arsenic by the stain produced on  $\text{HgBr}_2$  paper by  $\text{AsH}_3$ .
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- CHOUGHAK, D., *Ann. chim. anal. chim. appl.* [2], **4**, 138 (1922); *C. A.* **16**, 2278 (1922); *Analyst* **47**, 317 (1922). The colorimetric determination of arsenic by means of quinine molybdate.
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- ATKINS, W. R. G. and WILSON, E. G., *Biochem. J.* **20**, 1223 (1926); *C. A.* **21**, 1778 (1927). Colorimetric estimation of minute amounts of compounds of silicon, of phosphorus and of arsenic.

#### Benzoic Acid.

- JONES, A. J., *Pharm. J.* **115**, 144 (1925); *Analyst* **50**, 563 (1925); *C. A.* **20**, 95 (1926). Colorimetric determination of benzoic acid in cordials, etc.

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- PERLZWEIG, W. A. and BARRON, E. G., *Proc. Soc. Exptl. Biol. Med.* **24**, 233 (1926); *C. A.* **21**, 2284 (1927). New colorimetric method for determination of bile acids in blood.

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- VAN DEN BERGH, *Presse méd.* **29**, 441 (1921).
- PRESTON, J. *Lab. Clin. Med.* **11**, 879 (1926).
- HAWK, P. B. and BERGEIM, O., *Practical Physiological Chemistry*, 9 ed., pp.

396-397, P. Blakiston's Son and Co., Philadelphia, 1926. Determination of bile pigment in serum.

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SZILÁRD, P., Biochem. Z. **159**, 325 (1925). Colorimetric determination of bile salts in the blood.

LIFSCHÜTZ, I., Biochem. Z. **171**, 501 (1926). Determination of bile salts in blood. (Correction.) A criticism of Szilárd's method, Biochem. Z. **159**, 325 (1925).

SZILÁRD, P., Biochem. Z. **173**, 440 (1926); C. A. **21**, 753 (1927). Colorimetric determination of bile salts in blood.

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HASELHORT, G., Münch. med. Wochschr. **68**, 174 (1921); Ber. ges. Physiol. exptl. Pharmakol. **7**, 65 (1921). A new quantitative method for the determination of bilirubin in blood serum.

MEULENGRACHT, E., Compt. rend. soc. biol. **84**, 153 (1921); Ber. ges. Physiol. exptl. Pharmakol. **7**, 65 (1921). Quantitative bilirubin determinations in cases of bilirubinemia.

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### Biological Fluids.

ROHDE, A. and SWEENEY, M., J. Biol. Chem. **36**, 475 (1918). On a source of error in the use of picric acid in colorimetric estimation of biological fluids. Picric acid kept in a moist condition for several months must not be used.

### Bismuth.

THRESH, T. C., Pharm. J. **1880**, 641. Uses bismuth iodide method.

LEVY, L., Compt. rend. **103**, 1074, 1195; see also J. Anal. Chem. **1**, 201 (1887). Colored reactions of the rare mineral acids. Titanic, niobic, tantallic, stannic, arsenic, and vanadic acids, and bismuth oxide. Reagents used were either phenols or allied substances.

STONE, F. B., J. Soc. Chem. Ind. **6**, 416 (1887); see also J. Anal. Chem. **1**, 411 (1887). A delicate test for bismuth. Uses KI, which when added to  $\text{Bi}_2(\text{SO}_4)_3$  in dilute  $\text{H}_2\text{SO}_4$  gives a yellow color. 0.1 mg. Bi can be detected in 10 grams Cu.

PLANÈS, P., J. pharm. chim. **18**, 385 (1903); J. Chem. Soc. **86**, ii, 93 (1904); J. Soc. Chem. Ind. **22**, 1259 (1903). Method depends on the fact that in the presence of glycerol KI does not precipitate Bi but gives a yellow "solution." Conversely, the method can be used to estimate iodides. Standard made as follows: Dissolve 1 g. pure metallic Bi in 3 cc.  $\text{HNO}_3$  and 2.8 cc. water. Dilute to 100 cc. with glycerol. Dissolve 5 g. KI in 5 cc. of each of these



- solutions mixed and diluted to 50 cc. with a mixture of glycerol and water. About 1 per cent solutions of Bi are compared.
- CLOUD, T. C., *J. Soc. Chem. Ind.* **23**, 523 (1904). Determination of minute quantities of bismuth in copper and copper ores. Uses  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{HNO}_3$ , and KI. Will easily determine 0.01 mg. Bi. Method was used as a qualitative test for Bi by Abel and Field, *J. Chem. Soc.* **14**, 290 (1861).
- ROWELL, H. W., *J. Soc. Chem. Ind.* **27**, 102 (1908); *J. Chem. Soc.* **94**, ii, 325 (1908). Uses  $\text{H}_2\text{SO}_4$ , KI, and  $\text{H}_2\text{SO}_3$ . Large amounts of Pb, Cu, Sn, Sb, Au, and Ag must be absent.
- MOTHERWELL, H. A. B., *Eng. Mining J.* **104**, 1091 (1917); *C. A.* **12**, 460 (1918); *J. Soc. Chem. Ind.* **37**, 92A (1918).
- SPURGE, G., *Chem. Eng. Mining Rev.* **11**, 80 (1918); *C. A.* **13**, 2648 (1919); *Chem. Age* **4**, 584 (1921); *Analyst* **46**, 298 (1921). Colorimetric estimation of bismuth in high-grade ores. Details of procedure given in *C. A.* **13**, 2648 (1919).  $\text{HNO}_3$  solution of sample + KI + 3 drops  $\text{H}_2\text{SO}_3$ . 0.05 g. sample for 3 per cent Bi and over, 0.5 g. for below 3 per cent Bi. Comparative results with the  $\text{BiOCl}$  method show excellent agreement on ores containing 13 to 45 per cent Bi.
- PHILLIPS, W. T., *Eng. Mining J.* **105**, 882 (1918); *C. A.* **12**, 1446 (1918). Method practically the same as that reported by Motherwell [*Eng. Mining J.* **104**, 1091 (1917)] and has been in use in S. Wales for several years.
- SNELL, F. D., *Colorimetric Analysis*, p. 62, D. Van Nostrand Co., New York, **1921**. Determination of bismuth as the iodide.
- AUBRY, P., *J. pharm. chim.* **25**, 15 (1922); *C. A.* **16**, 2343 (1922); *Analyst* **47**, 129 (1922). Detection of bismuth in urine. Uses quinine sulfate,  $\text{H}_2\text{SO}_4$ , and KI. Orange-red precipitate. Gives a color at a dilution of 1 : 600,000 of  $\text{Bi}_2\text{O}_3$ . A direct colorimetric determination was not successful.
- CUNY, L. and POIROT, G., *J. pharm. chim.* [7], **28**, 215 (1923); *C. A.* **18**, 208 (1923); *Analyst* **49**, 48 (1924); *J. Soc. Chem. Ind.* **43**, B38 (1924). With P. Aubry's iodoquinic reagent [*J. pharm. chim.* **25**, 15 (1922)], addition of gum arabic causes an orange-yellow colloidal solution of the precipitate to form. Its color intensity varies with the Bi concentration.
- LAPORTE, C. E., *J. pharm. chim.* [7] **28**, 304 (1923); *C. A.* **18**, 1259 (1924); *J. Soc. Chem. Ind.* **43**, B 38 (1924). Method based on that of Aubry [*J. pharm. chim.* **25**, 15 (1922)] and the solubility of the precipitate in acetone. For a Bi content between 0.1 and 1 mg.  $\text{Bi}_2\text{O}_3$ , mix 10 cc. of the Bi solution in 10 per cent  $\text{HNO}_3$  with 2 cc. of Léger-Aubry's reagent, 8 cc. of acetone, and compare color with standard Bi solution similarly treated.
- AUTENRIETH, W. and MEYER, A., *Münch. med. Wochschr.* **71**, 601 (1924); *Chem. Zentr.* **1924**, ii, 220; *J. Chem. Soc.* **128**, i, 182 (1925). Determination of bismuth in organs, blood, and excreta. Method based on formation of  $\text{KBiI}_4$ .
- KÜRTHY, L. and MÜLLER, H., *Biochem. Z.* **147**, 377 (1924); *J. Chem. Soc.* **128**, ii, 73 (1925). Method suited to biological application. Bi precipitated as phosphate with  $(\text{NH}_4)_2\text{HPO}_4$  and the  $\text{PO}_4$  determined with molybdic acid, quinol and  $\text{Na}_2\text{CO}_3$ .

- KÜRTHY, L. and MÜLLER, H., *Biochem. Z.* **149**, 236 (1924).
- SCOTT, W. W., *Standard Methods of Chemical Analysis*, 4 ed., pp. 77 and 78, D. Van Nostrand Co., New York, **1925**. Uses cinchonine KI (method of W. C. Ferguson) and bismuth iodide (T. C. Thresh, *Pharm. J.* **1880**, 641) methods.
- HILL, C. A., *Lancet* **1925**, II, 1281; *C. A.* **20**, 1255 (1926).
- JONES, C. O. and FROST, E. C., *Ind. Eng. Chem.* **18**, 596 (1926). Note on the determination of small amounts of bismuth in copper. Use KI and  $\text{H}_2\text{SO}_4$ . Method suitable for the determination of 0.001 per cent of Bi in Cu.

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- MYERS, R. G., *J. Biol. Chem.* **41**, 119 (1920). A chemical study of the blood of several invertebrate animals. Colorimetric methods for different constituents.
- HAMMETT, F. S., *J. Biol. Chem.* **41**, 599 (1920). Studies of variations in the chemical composition of human blood.
- DALE, H. H. and EVANS, C. L., *J. Physiol.* **54**, 167 (1920-21). Colorimetric determination of the reaction of blood by dialysis.
- ATKINSON, H. V. and ETS, H. N., *J. Biol. Chem.* **62**, 5 (1922). Chemical changes of the blood under the influence of drugs. I. Ether. Use Kober nephelometer-colorimeter.
- DENIS, W. and HOBSON, S., *J. Biol. Chem.* **55**, 183 (1923). A study of the inorganic constituents of the blood serum in nephritis. Colorimetric methods given in *J. Biol. Chem.* **55**, 184.
- DENIS, W. (with the assistance of Julia Goddard), *J. Biol. Chem.* **56**, 473 (1923). A study of the inorganic constituents of the blood in experimental nephritis.
- KOEHLER, A. E., *J. Biol. Chem.* **62**, 816 (1923-24). Colorimetric determination of blood.
- WHELAN, M., *J. Biol. Chem.* **63**, 585 (1925). The effect of intravenous injection of inorganic chlorides on the composition of blood and urine.
- MILLER, A., LINDER, G. C. and VAN SLIKE, D. D., *J. Biol. Chem.* **64**, 625 (1925). The reducing substances of the blood. Compares several methods.
- LEWIS, H. B., with the cooperation of H. Updegraff, *J. Biol. Chem.* **65**, 187 (1925). The metabolism of sulfur. IX. The effect of repeated administration of small amounts of cystine. Creatinine and amino-acid N by Folin methods. Cystine by Looney's method. Blood by Folin-Wu procedure.
- STONE, W. J., *Blood Chemistry: Colorimetric Methods*, 2 ed., Paul B. Hoeber, Inc., New York. 129 pp. Reviewed in *J. Am. Med. Assoc.* **87**, 434 (1926).

### Blood Analysis.

- FOLIN, O. and WU, H., *J. Biol. Chem.* **38**, 81 (1919).
- MYERS, V. C., *Practical Chemical Analysis of Blood*, 2 ed., C. V. Mosby Co., St. Louis, **1924**.
- HOPKINS, E. G., MCCrackan, R. F. and SHARPE, W. F., *Bull. Med. Coll. Va.* **23**, No. 3, 24 (1926). Some time-saving procedures in the colorimetric analysis of

blood filtrates. I. Reliability of the dilution type of colorimeter. II. Preparation of permanent standards. III. Use of reduced amounts of blood and conservation of reagents. IV. Simplified calculations in colorimetry. "The diln. type of colorimeter is recommended for class use. Creatinine standards for the colorimetric detn. of creatinine are recommended in place of the dichromate standard. Creatinine as a by-product in the manuf. of meat juice is not now expensive. Folin's method of blood analysis was modified so that 4 constituents of clinical importance could be detd. in 2 cc. of blood. Calcs. in colorimetry were simplified so that results were obtained by multiplying readings by simple factors such as 10 or 2." L. W. Riggs, C. A. **21**, 2005 (1927).

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DUBIN, H., J. Biol. Chem. **33**, 377 (1918). Studies of the blood fat and lipoids of the dog before and after the production of experimental anemia. Nephelometric and colorimetric methods of Bloor.

HORIUCHI, Y., J. Biol. Chem. **44**, 345 (1920). Variation of the blood fat constituents of rabbits under normal conditions. Nephelometric methods for total fat and lecithin colorimetric for cholesterol.

### Boric Acid.

HÈBE BRAND, A., Z. Untersuch. Nahr. Genussm. **5**, 55 (1902); J. Soc. Chem. Ind. **21**, 278 (1902). Uses curcumin.

HÈBE BRAND, A., Z. Untersuch. Nahr. Genussm. **5**, 1044 (1902); Analyst **28**, 37 (1903). The amount of boric acid in fruits. Uses turmeric solution.

CASSAL, C. E. and GERRANS, H., Brit. Food J. **4**, 210 (1902) and Chem. News, **87**, 27 (1903); J. Chem. Soc. **84**, ii, 332 (1903); J. Soc. Chem. Ind. **22**, 381 (1903). Use an alcoholic solution of curcumin.

ALLEN, A. H. and TANKARD, A. R., Analyst **29**, 304 (1904). The determination of boric acid in cider, fruits, etc. Refer to the method of Cassal and Gerrans [Brit. Food J. **4**, 210 (1902)] for boric acid in milk and other foods. This method is based upon the fact that in the presence of oxalic acid the coloring matter of turmeric forms with boric acid an intense magenta-red color more delicate than the ordinary turmeric reaction (i.e., when oxalic acid is absent), and permanent for many hours.

CRIBB, C. H. and ARNAUD, F. W. F., Analyst **31**, 147 (1906); J. Chem. Soc. **90**, ii, 394 (1906). Approximate estimation of boric acid. Use turmeric paper.

BERTRAND G. and AGULHON, H., Compt. rend. **167**, 1433 (1913); J. Chem. Soc. **106**, ii, 146 (1914). Method of estimating extremely small quantities of boron in organic substances. Use turmeric paper.

FILIPPI, E., Arch. farmacognosia sci. affini. **3**, 29 (1914); through Ann. chim. applicata **1**, 564; C. A. **9**, 901 (1915). Determination of small quantities of boron in organic substances. Uses curcumin.

HALPHEN, G., Ann. fals. **8**, 1 (1915); J. Soc. Chem. Ind. **34**, 278; C. A. **9**, 2044 (1915). Uses turmeric solution.

HAWLEY, H., Proc. Soc. Pub. Anal. March 3, 1915; Chem. News **111**, 143 (1915). Routine detection and estimation of boric acid in butter. Uses an alcoholic extract of turmeric.

SNELL, F. D., *Colorimetric Analysis*, p. 126, D. Van Nostrand Co., New York, 1921. Determination of boric acid by curcumin.

SNELL, F. D., *Colorimetric Analysis*, p. 127, D. Van Nostrand Co., New York, 1921. Determination of boric acid by turmeric paper.

**Boron.** (See **Boric acid**.)

**Bromine.**

HALL, C. C., *J. Anal. Chem.* **4**, 167 (1890). Rapid determination of bromine in presence of chlorine. Br liberated by Cl and dissolved in  $\text{CHCl}_3$ .

DIBDIN, W. J. and COOPER, L. H., *Analyst* **35**, 159 (1910); *J. Chem. Soc.* **98**, ii, 448 (1910); *C. A.* **4**, 2249 (1910); *J. Soc. Chem. Ind.* **29**, 562 (1910). Colorimetric estimation of small quantities of bromine in the presence of large quantities of chlorine and small quantities of iodine. Use  $\text{H}_2\text{SO}_4$  and Cl. Brown color of Br solution is matched against a standard similarly treated.

BAUBIGNY, H., *Bull. soc. chim.* **9**, 352; *C. A.* **5**, 2380 (1911). Determination of very small amounts of bromine in the presence of chlorides and iodides. Recommends the colorimetric method for amounts of the order of 0.01 mg. but for 1 mg. or more, the Br is distilled off after adding  $\text{KMnO}_4$  and weighed as  $\text{AgBr}$ .

*J. Assocn. Official Agri. Chem.* [I] **1**, 97 (1915). A study of the colorimetric methods for Br shows that they are not entirely satisfactory.

SWEENEY, O. R. and WITHROW, J. R., *J. Ind. Eng. Chem.* **9**, 671 (1917). The chemical examination of natural brines. Br liberated by Cl-water and dissolved in  $\text{CCl}_4$ .

OPPENHEIMER, E., *Arch. exptl. Path. Pharmacol.* **89**, 17 (1921); *J. Chem. Soc.* **120**, ii, 273 (1921); *Analyst* **46**, 516 (1921). A new method for the estimation of bromine in very small quantities. Schiff's reagent is colored deep blue violet by bromine.

SCOTT, W. W., *Standard Methods of Chemical Analysis*, 4 ed. p. 96, D. Van Nostrand Co., New York, 1925. Uses magenta reagent. A solution containing 0.001 g. Br. per liter gives a violet to reddish-violet color.

**Brucine.**

DOWZARD, E., *Proc. Chem. Soc. (London)*, **18**, 220 (1902); *Chem. News* **87**, 99 (1903); *J. Soc. Chem. Ind.* **21**, 1560 (1902). The determination of brucine in *nux vomica*.

WÖBER, A., *Z. angew. Chem.* **31**, i, 124 (1918); *J. Chem. Soc.* **114**, ii, 339 (1918); *J. Soc. Chem. Ind.* **37**, 441A (1918); *Z. anal. Chem.* **62**, 256 (1923). Colorimetric estimation of brucine in presence of strychnine. Uses a mixture of concentrated  $\text{HNO}_3$  and 20 per cent  $\text{H}_2\text{SO}_4$  in Dowzard's method and adds a saturated solution of  $\text{KClO}_3$  immediately after the reaction. This modified procedure is more reliable than the original in which the coloration fades with varying velocity.

**Buffer.**

McILVAINE, T. C., *J. Biol. Chem.* **49**, 183 (1921). Buffer solution for colorimetric comparison.

**Butyric Acid.**

- DENIGÈS, G., Bull. soc. pharm. Bordeaux, **1917**, No. 3; Répert. pharm. **28**, 262 (1917); Ann. chim. anal. **23**, 27 (1918); J. Chem. Soc. **114**, ii, 138 (1918); C. A. **11**, 3198 (1917). Detection and estimation of butyric acid.

**Calcium.**

- HINDS, J. I. D., J. Am. Chem. Soc. **18**, 661 (1896) and Chem. News **73**, 285, 299 (1896). Photometric method for the quantitative determination of lime and sulfuric acid.
- HINDS, J. I. D., J. Am. Chem. Soc. **22**, 269 (1900). Lime and sulfuric acid by the photometric method.
- LYMAN, H., J. Biol. Chem. **21**, 551 (1915). A turbidimetric method. Ca is precipitated as  $\text{CaC}_2\text{O}_4$ , dissolved, and reprecipitated as Ca-soap. The cloud thus formed is matched in a colorimeter against a standard suspension.
- HOWLAND, J., HAESSLER, F. H. and MARRIOTT, W. MCK., Proc. Amer. Soc. Biol. Chem., J. Biol. Chem. **24**, xviii (1916). The use of a new reagent for microcolorimetric analysis as applied to the determination of calcium and of inorganic phosphates in the blood serum. The methods are based on the fact that the red color of a solution of  $\text{Fe}(\text{CNS})_3$  is discharged by certain substances, e.g., oxalates and phosphates. Ca is precipitated as the oxalate, dissolved in acid, added to a standard solution of  $\text{Fe}(\text{CNS})_3$ , and made up to a definite volume. The color of the solution is compared with that of a solution containing known amounts of  $\text{CaC}_2\text{O}_4$  and  $\text{Fe}(\text{CNS})_3$ . The phosphates are precipitated as  $\text{MgNH}_4\text{PO}_4$ , the latter dissolved and color comparison made as above.
- MARRIOTT, W. MCK. and HOWLAND, J., J. Biol. Chem. **32**, 233 (1917). A micro method for the determination of calcium and magnesium in blood serum. Use  $\text{Fe}(\text{CNS})_3$  method.
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- CLARK, G. W., J. Biol. Chem. **49**, 487 (1921). The micro determination of calcium in whole blood, plasma, and serum by direct precipitation. Nephelometrically as Ca-soap. Colorimetrically with ferric thiocyanate.
- LAIDLAW, P. P. and PAYNE, W. W., Biochem. J. **16**, 494 (1922); C. A. **17**, 3848 (1923); J. Soc. Chem. Ind. **41**, 918A (1922). Use alizarinate method. Accurate to 0.002 mg. Ca.
- GRÉGOIRE, A., CARPIAUX, E., LAROSE, E., and SOLA, T., Bull. soc. chim. Belg. **32**, 123 (1923); C. A. **17**, 1931 (1923); J. Soc. Chem. Ind. **42**, 427A (1923); Z. anal. Chem. **64**, 342 (1924). Ca precipitated as pale yellow colloidal Ca oleate.
- CHÉNEVEAU, C. and BOUSSU, R., Compt. rend. **177**, 1296 (1923). Sur le dosage du calcium par la méthode opacimétrique.
- FEIGL, F. and PAVELKA, F., Mikrochemie **2**, 85 (1924); J. Chem. Soc. **126**, ii, 784 (1924). Use ammonium ferrocyanide and 50 per cent alcohol.

ROE, J. H. and KAHN, B. S., J. Biol. Chem. **67**, 585 (1926). A colorimetric method for the estimation of blood calcium.

#### Calculations.

STONER, W. H., J. Lab. Clin. Med. **10**, 574 (1924-25). Simplified colorimetric calculation.

HOPKINS, E. G., McCrackan, R. F., and SHARPE, W. F., Bul. Med. Coll. Va. **23**, No. 3, 24 (1926); C. A. **21**, 2005 (1927).

McCrackan, R. F., PASSAMANECK, E., and HARMAN, (Miss) K. E., J. Chem. Education **3**, 416 (1926). Simplified calculations in colorimetry.

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HALE, C. F., Chem. Eng. **14**, 475; C. A. **6**, 666 (1912). Colorimetric test for caramel.

MEADE, G. P., Ind. Eng. Chem. **15**, 275 (1923). The estimation of caramel in sugar products—criticism of the Ehrlich method.

#### Carbohydrate. (See also, **Dextrose, Glucose, Lactose, and Sugar.**)

NEITZEL, E., Neue Z. Rübenzucker Ind. **10**, 833; J. Soc. Chem. Ind. **13**, 285 (1894).

NEITZEL, E., Z. Spiritusind. **17**, 163 (1894); J. Soc. Chem. Ind. **13**, 985 (1894).

NEITZEL, E., Z. Spiritusind. **20**, 163 (1896); Dingler's polytech. J. **297**, 164; Z. anal. Chem. **35**, 600 (1896); Chem. News **74**, 293 (1896). Method patented!

WACKER, L., Ber. **42**, 2675 (1909); C. A. **3**, 2575 (1909); cf. C. A. **2**, 175 (1908). Colorimetric method for the determination of mol. wt. of carbohydrates. (Distinction of primary from secondary and tertiary alcohols.)

DEHN, W. M., and HARTMAN, F. A., J. Am. Chem. Soc. **36**, 403 (1914). Use picric acid.

BERNHARD, A., Diss. Brooklyn Polytech. Inst., June, 1915; Sugar **17**, No. 11, 41 (1915); C. A. **10**, 1230 (1916). A simple colorimetric method for the determination of free reducing sugar and total carbohydrates in miscellaneous food materials. Uses the color reaction produced by heating dextrose in alkaline solution with picric acid. Picramic acid formed.

DISCHE, Z. and POPPER, H., Klin. Wochschr. **5**, 1973 (1926); C. A. **21**, 431 (1927). Colorimetric micromethod for estimating the total carbohydrate content of organs and body fluids.

DISCHE, Z. and POPPER, H., Biochem. Z. **175**, 371 (1926); C. A. **21**, 932 (1927); cf. C. A. **21**, 431. A new colorimetric microchemical determination of carbohydrates in organs and body fluids.

#### Carbon.

EGGERTZ, Jern Kontorets Annaler, **1862**, p. 54. Colorimetric estimation of carbon in iron.

EGGERTZ, Chem. News **7**, 254 (1863). Methods of estimating carbon in iron and steel. Reports several methods, including a colorimetric one based on the yellow to brown colored solution obtained by dissolving the sample in HNO<sub>3</sub>.

- EGGERTZ, Chem. News **20**, 65 (1869); Dingler's polytech. J. **194**, 116. Process for determining the carbon chemically combined with iron.
- DINGLER, Jahrb. der Kaiserlich-Königlichen Geologischen Reichsanstalt No. 4, **1869**; Chem. News **21**, 131 (1870). Colorimetric carbon-test of Eggertz.
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VIRGILI, J. F., *Ann. chim. anal. chim. appl.* **14**, 85 (1909); *C. A.* **3**, 1737 (1909); *Chem.-Ztg.* **32**, 1254 (1908). Uses aniline chloride in HCl. Violet changing to blue obtained with  $\text{ClO}_3$  and certain other oxidizing agents.

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- PHELPS, E. B., Bull. No. 1, Ohio State Board of Health, Jan. 1913. Uses orthotolidine dissolved in dilute HAc as a delicate color test for free chlorine in water.
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MAURIAC, P. and DEFAVE, *Compt. rend. soc. biol.* **73**, 143 (1912); *C. A.* **8**, 1133 (1914). Clinical colorimetric methods for determining cholesterol.

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HENES, E., Jr., *Proc. N. Y. Path. Soc.* **13**, 155 (1913). Uses the extraction method of Weston and Kent and the colorimetric method of Grigaut (*Compt. rend. soc. biol.* **68**, 827 (1910)).

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- DETONI, G. M., *J. Biol. Chem.* **70**, 207 (1926).

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**Chromium.**

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- HOGG, T. W., *J. Soc. Chem. Ind.* **14**, 1022 (1895). Uses Eggertz' method for C and his method for Cr described in *J. Soc. Chem. Ind.* **10**, 340 (1891).
- HILLEBRAND, W. F., *J. Am. Chem. Soc.* **20**, 454 (1898). The colorimetric estimation of small amounts of chromium with special reference to the analysis of rocks and ores. Cr transformed into  $\text{Na}_2\text{CrO}_4$ , solution made slightly alkaline with  $\text{Na}_2\text{CO}_3$  and diluted to a definite volume. Compared with a standard solution.
- CAZENEUVE, M., *Bull. soc. chim.* **29**, 758; *Chem. News* **89**, 268 (1904). Describes the color reaction produced when diphenylcarbazide is added to chromic acid or a chromate. A very intense purple-violet color is thus produced. Reaction very sensitive.
- MOULIN, A., *Bull. soc. chim.* **31**, 295 (1904); *Chem. News* **89**, 268 (1904); *J. Chem. Soc.* **86**, ii, 368 (1904); *J. Soc. Chem. Ind.* **23**, 457 (1904). The author has applied the color (purple) reaction between diphenylcarbazide and chromic acid observed by Cazeneuve to the determination of Cr. Solution prepared by dissolving 2 g. of the substance in 100 cc. ( $90^\circ$ ), to which 10 cc. of HAc have been added, and diluting to 100 cc. with alcohol. Standard Cr solution contains 0.05 mg. of the acid per cubic centimeter. The Cr compound is first converted into  $\text{K}_2\text{CrO}_4$  by  $\text{H}_2\text{O}_2$  in presence of KOH and the solution finally neutralized with HAc. Precautions: (1) Use excess of diphenylcarbazide solution. (2) Chromate (sample) must be approximately the same strength as the standard chromic acid solution.
- HORN, D. W., *Am. Chem. J.* **35**, 253 (1906). Variable sensitiveness in the colorimetry of chromium. H. points out that the sensitiveness is a variable and that the maximum sensitiveness must be determined for each colorimetric method.
- KOENIG, P., *Landw. Jahrb. Schweiz.* **39**, 775 (1910); *J. Chem. Soc.* **100**, ii, 524 (1911); *Chem. Zentr.* **1**, 498 (1911); *J. Soc. Chem. Ind.* **30**, 297 (1911). Uses disodium 1 : 8-dihydroxynaphthalene-3 : 6-disulphonate for determining minute quantities of Cr in plant ash.
- MEYERFELD, J., *Chem.-Ztg.* **34**, 948; *C. A.* **4**, 3178 (1910). Pyrogallol dimethyl ether, a sensitive reagent for chromic acid, ferric salts and nitrous acid. 0.008 mg.  $\text{KNO}_3$  in 5 cc. gives a yellow coloration with a water solution of pyrogallol dimethyl ether (1 : 50). Very dilute solutions of Cl or Bi give yellow colorations. The solution of the reagent must be freshly prepared as on standing it becomes yellow.
- KOENIG, P., *Chem.-Ztg.* **35**, 277 (1911); *J. Chem. Soc.* **100**, ii, 337 (1911). Uses a water solution of di-Na 1 : 8-dihydroxynaphthalene-3 : 6-disulphonate. Yields a red or violet coloration with chromic acid, chromates, or dichromates. Will detect 0.0008 mg. Cr. Can be used in presence of Fe by adding  $\text{H}_3\text{PO}_4$  to destroy the green color due to Fe.
- GARRATT, F., *J. Ind. Eng. Chem.* **5**, 298 (1913); *C. A.* **7**, 2029 (1913); *J. Soc.*

- Chem. Ind. **32**, 490 (1913); Z. anal. Chem. **63**, 351 (1923). A colorimetric method for the determination of chromium in steel. Method based upon a pink to cherry-red color with  $\text{CrO}_3$  and disodium 1: 8-dihydroxynaphthalene-3: 6-disulfonate (Koenig's reagent, cf. C. A. **5**, 2046) in solutions acid with  $\text{H}_2\text{SO}_4$  and  $\text{H}_3\text{PO}_4$ . 0.001 per cent Cr can readily be detected. Method recommended for materials containing less than 0.6 per cent Cr.
- DITTRICH, M., Z. anorg. Chem. **80**, 171 (1913); J. Chem. Soc. **104**, ii, 344 (1913); J. Soc. Chem. Ind. **32**, 383 (1913). The estimation of small quantities of manganese and chromium in minerals and rocks. Uses  $\text{NH}_4$  persulfate and silver for Mn and chromate method for Cr, after removal of Ag with NaCl.
- VAN ECK, P. N., Chem. Weekblad **12**, 6 (1915); C. A. **9**, 769 (1915). Sensitive reactions of chromates. Five reactions are given. Some may be used to estimate the quantity of Cr. Disodium 1: 8-dihydroxynaphthalene-3: 6-disulfonate is the most sensitive; a deep red color is obtained with 0.0008 mg. of Cr.
- APPELBAUM, A. I., Chem. Analyst **27**, 7 (1918); C. A. **12**, 1952 (1918). Oxidizes with NaOH and  $\text{Na}_2\text{O}_2$  and adds  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{SO}_4$  and disodium 1: 8-dihydroxynaphthalene-3: 6-disulfonate.
- HACKL, O., Chem.-Ztg. **44**, 63 (1920); C. A. **14**, 1946 (1920). Detection and estimation of very small quantities of chromium in minerals and ores containing silicates and carbonates.
- SNELL, F. D., Colorimetric Analysis, p. 70, D. Van Nostrand Co., New York, **1921**. Determination of chromium as the chromate.
- SNELL, F. D., Colorimetric Analysis, p. 71, D. Van Nostrand Co., New York, **1921**. Determination of chromium by disodium 1: 8-dihydroxynaphthalene-3: 6-disulfonate.
- EVANS, B. S., Analyst **46**, 38 (1921); C. A. **15**, 1264 (1921); Chem. News **121**, 297 (1920); J. Soc. Chem. Ind. **40**, 181A (1921). Cr oxidized by  $\text{KMnO}_4$  to chromate solution, made acid, and the bichromate compared with standard  $\text{K}_2\text{Cr}_2\text{O}_7$  solution.
- EVANS, B. S., Analyst **46**, 285 (1921); C. A. **15**, 3047 (1921); cf. C. A. **15**, 1264; J. Soc. Chem. Ind. **40**, 627A (1921). The estimation of small amounts of chromium in steel. Chromic acid and diphenylsemicarbazide [cf. Cazeneuve, Analyst **25**, 331 (1900)] give an intense purple color similar to that of permanganate. Reagent: 1 g. of diphenylsemicarbazide in 10 cc. AcOH and diluted to 1000 cc. with water. Procedure: 5 cc. of the reagent and 10 cc. of 25 per cent  $\text{H}_2\text{SO}_4$  (by vol.) are added to the solution to be tested. Solutions containing 4 g. of dissolved electrolytic iron gave the same colors as similar solutions containing no iron. Cr content varied between 0.004 and 0.0017 per cent. Accurate to 0.0001 per cent.
- SCOTT, W. W., Standard Methods of Chemical Analysis, 4 ed., pp. 162 and 163, D. Van Nostrand Co., New York, **1925**. Uses the dichromate (Cr oxidized by  $(\text{NH}_4)_2\text{S}_2\text{O}_8$ ,  $\text{NH}_4\text{NO}_3$ , and  $\text{AgNO}_3$ ) and diphenyl carbazide methods.
- SNODDY, A. O., J. Oil & Fat Ind. **2**, 20 (1925); C. A. **20**, 118 (1926). The detection and estimation of small amounts of chromium in fats. Uses diphenylcarbazine.



**Citral.**

- CHACE, E. M., J. Am. Chem. Soc. **28**, 1472 (1906); J. Soc. Chem. Ind. **25**, 1116 (1906). A method for the determination of citral in lemon oils and extracts. Uses Schiff's reagent.
- HILTNER, R. S., J. Ind. Eng. Chem. **1**, 798 (1909). A method for the determination of citral in lemon extracts and lemon oils. Uses 1 per cent solution of metaphenylenediamine hydrochloride in 50 per cent ethyl alcohol. Decolorizes with fuller's earth or animal charcoal.
- LITTLE, L. D., J. Am. Pharm. Assocn. **3**, 553; C. A. **8**, 1851 (1914). A colorimetric method for the determination of citral in extracts of lemon and in oil of lemon. Uses 0.200 g. diaminophenol-HCl (amidol) dissolved in 100 cc. of 65 per cent (by vol.) alcohol. For a standard citral solution uses 0.001 g. of pure citral in 1 cc. of 50 per cent alcohol. Manipulation is similar to the Chace method except that it can be carried out at room temperature.
- LITTLE, L. D., Am. Perfumer **9**, 74 (1914); Z. anal. Chem. **55**, 217 (1916).
- PARKER, C. E. and HILTNER, R. S., J. Ind. Eng. Chem. **10**, 608 (1918); cf. U. S. Dept. Agr. Bureau of Chemistry, Bull. **122**, 34; **132**, 102; **137**, 70. An improved method for determining citral. A modification of the Hiltner method. Uses  $\text{H}_2\text{C}_4\text{O}_4$  to inhibit the production of a blue or green coloration which sometimes appears in the *m*-phenylenediamine hydrochloride method. The usual yellow color is obtained.

**Citric Acid.**

- DENIGÈS, G., Compt. rend. soc. biol. **54**, 197 (1902).

**Cobalt.**

- LAMPADIUS, W. A., J. prakt. Chem. **13**, 385 (1838); Z. anal. Chem. **5**, 425 (1866). Colorimetric determination of small amounts of cobalt in minerals, etc. An approximate method based upon the reddish-brown color produced when  $\text{NH}_4\text{OH}$  is added to cobalt salt solutions.
- WAGNER, R., J. prakt. Chem. **61**, 129 (1854); Z. anal. Chem. **5**, 425 (1866).
- WINKLER, C., J. prakt. Chem. **97**, 414 (1866); Z. anal. Chem. **5**, 425 (1866).
- KNIEDER, Chem. Zentr. **1894**, ii, 452; Rev. minera, met. ing. **1893**, 398. Colorimetric assay of cobalt ores. Forms  $\text{CoCl}_2$  and compares with a series of standards.
- CHALLINOR, R. W., J. Roy. Soc., New South Wales, **38**, 406 (1905); J. Chem. Soc. **94**, ii, 988 (1908). Approximate colorimetric estimation of cobalt and nickel in the presence of each other. Ni and Co obtained jointly by elec. Then dissolve in  $\text{HNO}_3$  (1 : 1), evaporate almost to dryness and dilute to a definite volume. Solution green = more than 76 per cent Ni, solution pink = more than 24 per cent Co. An aliquot part is used with standard Co or Ni solution.
- MELLOR, J. W., Trans. Ceram. Soc. England **8**, 132; C. A. **4**, 1440 (1910). Colorimetric determination of cobalt in presence of nickel. Method based upon the fact that ether or amyl alcohol take up Co with blue coloration, leaving Ni in water.

- HÜTTNER, C., *Z. anorg. Chem.* **86**, 341 (1914); *J. Soc. Chem. Ind.* **33**, 614 (1914); *Z. anal. Chem.* **54**, 471 (1915). Colorimetric determination of cobalt, nickel, iron, and copper. Matches HCl solutions of the chlorides. *C. A.* **8**, 2540 (1914) gives a long abstract of the paper.
- ATAK, F. W., *J. Soc. Chem. Ind.* **34**, 641 (1915); *C. A.* **9**, 2363 (1915). Uses  $\alpha$ -nitroso- $\beta$ -naphthol.
- POWELL, A. D., *J. Soc. Chem. Ind.* **36**, 273 (1917); *C. A.* **11**, 1801 (1917). Co extracted by amyl alcohol from its solution in  $\text{NH}_4\text{CNS}$ . Blue solution obtained.  $\text{NH}_4\text{CNS}$  solution must be at least 25 per cent.
- JONES, E. G., *Analyst* **43**, 317 (1918); *C. A.* **13**, 15 (1919); *J. Soc. Chem. Ind.* **37**, 630A (1918). Uses  $\alpha$ -nitroso- $\beta$ -naphthol as employed by F. W. Atak. *J. Soc. Chem. Ind.* **34**, 641 (1915).
- SNELL, F. D., *Colorimetric Analysis*, p. 76, D. Van Nostrand Co., New York, **1921**. Determination of cobalt as the chloride in concentrated HCl.
- SNELL, F. D., *Colorimetric Analysis*, p. 76, D. Van Nostrand Co., New York, **1921**. Determination of cobalt by  $\alpha$ -nitroso- $\beta$ -naphthol.
- BRALEY, S. A. and HOBART, F. B., *J. Am. Chem. Soc.* **43**, 482 (1921); *Chem. News* **122**, 243 (1921); *Analyst* **46**, 300 (1921); *J. Soc. Chem. Ind.* **40**, 327A (1921). A new method for the detection and estimation of cobalt. Uses dimethylglyoxime, HAc and NaAc. Brown coloration which is not discharged by mineral acids.
- HACKL, O., *Chem.-Ztg.* **46**, 385 (1922); *C. A.* **16**, 2280 (1922). Detection and determination of small quantities of nickel and cobalt in silicate rocks. Ni by dimethylglyoxime. Co by  $\alpha$ -nitroso- $\beta$ -naphthol or Vogel's test with thiocyanate.
- AUGER, V. and ODINOT, L., *Compt. rend.* **178**, 710 (1924); *C. A.* **18**, 1798 (1924); *J. Soc. Chem. Ind.* **43**, B318 (1924). Method is based upon the fact that solutions of Co salts in the presence of a large excess of HCl give a blue color which can be compared with standards and the Co thus determined. The color varies with the concentration of the acid, becoming more intense with greater amounts of HCl. The acid must be free from iron.
- NICHOLS, M. L. and COOPER, S. R., *J. Am. Chem. Soc.* **47**, 1268 (1925); *C. A.* **19**, 1833 (1925). New qualitative tests for copper, iron, and cobalt. A saturated solution of dinitrosoresorcinol will detect as little as 0.004 mg. of Cu and somewhat less Fe and Co in 1 cc. of neutral solution.
- DENIGÈS, G., *Compt. rend.* **180**, 1748 (1925). A new spectroscopic and colorimetric method for the detection and immediate determination of cobalt. "If as little as 0.04 mg. of Co is present per cc. of soln., 0.1 cc. of the latter mixed with 5 cc. of concd. HCl gives a blue color and the soln. shows characteristic absorption bands when viewed with a direct-vision spectroscope. By photographing the spectrum or by comparing the depths of color it is possible to det. the Co content with little difficulty. Other ions giving colored chlorides interfere somewhat with the test. If a little  $\text{SnCl}_2$  is added to the soln., the interference of  $\text{Cu}^{++}$  and  $\text{Fe}^{+++}$  is prevented. The reaction is useful for the detection of Co in Ni salts." W. T. H., *C. A.* **19**, 2615 (1925).

EVANS, B. S., *Analyst* **50**, 389 (1925). "The method is based upon the fact that a highly colored cobaltamine results when Co in ammoniacal soln. is treated with an oxidizing agent. The interference of Ni is prevented by adding Na citrate and keeping the  $\text{NH}_3$  concn. low. To det. Co in steel, dissolve 5 g. of metal in aqua regia. Nearly neutralize the resulting soln. with  $\text{NH}_3$  and ppt. Fe by adding ZnO suspension. Make up to 500 cc., mix and filter. Take 150 cc. of filtrate, add 22.5 cc. of 7 N  $\text{NH}_4\text{OH}$ , 7.5 cc. of 20 per cent  $\text{NH}_4\text{Cl}$  soln. and about 0.6 g. of  $\text{Na}_2\text{O}_2$ . After the  $\text{MnO}_2$  ppt. has settled, filter, add 5 g. of Na citrate and exam. the filtrate in the colorimeter." W. T. H., C. A. **19**, 3073 (1925).

### Colchicine.

FABINYI, R., *Oesterr. Chem. Ztg.* **15**, 61; C. A. **6**, 1340 (1912); J. Soc. Chem. Ind. **31**, 298 (1912). Colorimetric determination of morphine and colchicine. The colchicine determination is based on the dark olive-green color resulting upon boiling the solution and adding  $\text{FeCl}_3$ .

FABINYI, R., *Verb. Ges. deut. Naturforsch. Aerzte*, **1912**, ii, 1, 230; J. Chem. Soc. **102**, ii, 503 (1912). Colorimetric estimation of colchicine. Boils with a little HCl and adds 0.5 cc. of 1 per cent  $\text{FeCl}_3$ . Dark green coloration develops. One hour in darkness required for maximum intensity to develop.

### Colloids.

ROHLAND, P., *Z. anal. Chem.* **52**, 657 (1913); C. A. **7**, 4030 (1913). A colorimetric method for the determination of colloidal material in sewage.

### Colorimeters.

HERAPATH, T. J., *J. prakt. Chem.* **56**, 255 (1852); *ibid.* **60**, 242 (1853); J. Chem. Soc. **5**, 27 (1853).

MÜLLER, A., *J. prakt. Chem.* **60**, 474 (1853); *ibid.* **66**, 193 (1855); *Z. anal. Chem.* **5**, 423 (1866).

STAMMER, C., *Dingler's polytech. J.* **159**, 341 (1861); see also *Z. anal. Chem.* **3**, 227 (1864). Calls his instrument a "chromoscope."

SIEMENS and HALSKE, *Z. anal. Chem.* **3**, 218 (1864).

DEHMS, F., *Dingler's polytech. J.* **173**, 436 (1864); *Z. anal. Chem.* **3**, 218, 494 (1864).

MILLER, F. B., *J. Chem. Soc.* **18**, 118 (1865).

COLLARDEAU, *Z. anal. Chem.* **5**, 423 (1866).

HOUTTON and LABILLARDIÈRE, *Dingler's polytech. J.* **27**, 54; *Z. anal. Chem.* **5**, 423 (1866).

MORTON, *Chem. News* **21**, 31 (1870); *Z. anal. Chem.* **9**, 473 (1870). Gives a description and illustrative cuts of Duboscq's colorimeter.

BRITTON, J. B., *J. Franklin Inst.* **89**, 356 (1870).

RHEINECK, H., *Dingler's polytech. J.* **201**, 433 (1871); *J. Chem. Soc.* **24**, 1223 (1871).

SALLERON, *LES MONDES*, Nov. 30, 1871; *Chem. News* **24**, 276 (1871); *Dingler's polytech. J.* **203**, 141 (1872); *J. Chem. Soc.* **25**, 527 (1872).

- STAMMER, C., *Dingler's Polytech. J.* **203**, 137 (1872); *J. Chem. Soc.* **25**, 527 (1872). An improved form of S.'s chromoscope. Set of colored glass standards used.
- HARVEY, S., *Chem. News* **27**, 262 (1873). Describes a colorimeter consisting of two graduated tubes fastened in a rack with a swinging mirror below.
- DAVIS, G. E., *Chem. News* **27**, 299 (1873).
- KING, J. F., *Chem. News* **31**, 133 (1875); *J. Chem. Soc.* **28**, 1052 (1875). Process for the estimation of color in water. Uses glass tubes 15 inches long and of such diameter that when filled to within 3 inches of the top, they contain exactly 8 ounces of water.
- HEHNER, O., *Chem. News* **33**, 185 (1876); *J. Chem. Soc.* **30**, 326 (1876). Uses graduated cylinders, having glass taps fused into their sides so that the solution may be run out of either till color matches.
- MILLS, E. J., *Proc. Chem. Soc.* April 14, 1877; *Chem. News* **35**, 161 (1877). Two tubes each fitted with a loosely fitting disk of white or black glass (as may be required) which can be moved up and down by means of a glass rod fitted (water-tight) at the bottom of the tube.
- BOTTOMLEY, J., *Chem. News* **38**, 191 (1878). Similar to E. J. Mills' apparatus.
- GÜNSBERG, R., *Dingler's polytech. J.* **228**, 457 (1878).
- LEEDS, A. R., *Chem. News* **37**, 229 (1878); *J. Chem. Soc.* **34**, 807 (1878).
- WOLFF, C. H., *Chem.-Ztg.* No. 47, **1879**; *Chem. News* **40**, 277 (1879); *Pharm. Zig.* **24**, 587; *Z. anal. Chem.* **18**, 337 (1879). A modified Dubosq instrument.
- PUSCH, *Arch. Pharm.* **214**, 227 (1879).
- WOLFF, C. H., *Correspondenz-blatt des Vereines Anal. Chem.* No. 2, Jan. 15, 1880; referred to in *Chem. News* **41**, 109 (1880). See original on account of the illustrations there given.
- BAYLEY, T., *Chem. News* **41**, 170 (1880); *Z. anal. Chem.* **19**, 470 (1880). Copper by means of the "reflection cuprimeter."
- CROOKES, W., ODLING, W. and TIDY, C. M., *Chem. News* **43**, 174 (1881). Describes a hollow wedge type colorimeter.
- CROOKES, W., ODLING, W. and TIDY, C. M., *Chem. News* **45**, 170 (1882).
- STEAD, J. E., *Chem. News* **47**, 285 (1883); *J. Chem. Soc.* **44**, 1032 (1883).
- GIANNETTI, G., *Gazz. chim. ital.* **16**, 65 (1886); *J. Chem. Soc.* **50**, 738 (1886). Describes a colorimeter. Images of standard and "unknown" are projected on a mirror side by side.
- RIDSDALE, C. H., *J. Soc. Chem. Ind.* **5**, 585 (1886); see also *J. Anal. Chem.* **1**, 221 (1887).
- LOVIBOND, J. W., *Eng. Pat.* 12,867, Oct. 9, 1886; *J. Soc. Chem. Ind.* **6**, 522 (1887). Improvements in apparatus for standardizing and measuring intensity of color in transparent bodies.
- STOKES, A. W., *Proc. Chem. Soc.*, Dec. 15, 1887; *Chem. News* **56**, 275 (1887).
- LOVIBOND, T. W., *J. Soc. Chem. Ind.* **7**, 69 (1888). Exhibited J. W. Lovibond's apparatus.
- LOVIBOND, J. W., *J. Soc. Chem. Ind.* **7**, 424 (1888). A description of the tintometer, with some remarks on its application to chemical analysis.

- RIDSDALE, C. H., *J. Soc. Chem. Ind.* **7**, 70 (1888); see also *J. Anal. Chem.* **3**, 176 (1889).
- FRESENIUS, W., *Z. anal. Chem.* **28**, 686 (1889). A brief mention of J. W. Lovibond's tintometer and also M. Müller's colorimeter for Nesslerizing water and of a A. Jolle's colorimeter.
- LOVIBOND, J. W., *J. Soc. Chem. Ind.* **9**, 10 (1890); *J. Chem. Soc.* **58**, 1461 (1890).
- GALLENKAMP, *Chem.-Ztg. Rep.* **15**, 324 (1891); *J. Soc. Chem. Ind.* **11**, 547 (1892); *Chem. News* **68**, 37 (1893); *Z. anal. Chem.* **32**, 206 (1893).
- PELLET, H. and DEMICHEL, A., *Bull. de l'assoc. belge des chimistes* **5**, 530; *Z. anal. Chem.* **31**, 432 (1892).
- KRÜSS, H., *Z. anorg. Chem.* **5**, 325 (1894); *J. Chem. Soc.* **66**, ii, 158 (1894); *Z. anal. Chem.* **34**, 60 (1895).
- PULFRICH, C., *Z. Instrumentenk.* **14**, 210; *Z. anal. Chem.* **34**, 446 (1895).
- KRÜSS, H., *Z. Instrumentenk.* **14**, 283; *Z. anal. Chem.* **34**, 745 (1895). Compares K.'s colorimeter with the similar one described by Pulfrich (*Z. Instrumentenk.* **14**, 210).
- PROCTER, H. R., *J. Soc. Chem. Ind.* **14**, 122 (1895).
- WHITSON, A. R., *Bull. No. 85, Wis. Agr. Expt. Sta.* (1901). See also *Bull. No. 93*.
- SCHREINER, O., *J. Am. Chem. Soc.* **25**, 1058 (1903). On a colorimetric method for the estimation of phosphates in the presence of silica. Used a colorimeter similar to the one described and illustrated by Whitson (*Bull. 85, Wisc. Agr. Expt. Sta.*).
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- MEISLING, A. A., *Z. anal. Chem.* **43**, 137 (1904).
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- SMEATON, W. G., *J. Am. Chem. Soc.* **28**, 1433 (1906).
- WHITE, C. H., *Bull. Am. Inst. Mining Eng.* **11**, 743 (1906).
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- PLESCH, J., *Z. Chem. Apparat*, **3**, 212; *C. A.* **2**, 3295 (1908). Chromophotometer. Used for the determination of color concentration and the relative proportions in a mixture of a number of colors.
- BRATKOWSKI, W., *Z. Farben-Ind., through Chem. Zentr.*, **II**, 95 (1908); *C. A.* **3**, 1134 (1909).

- STEIGER, G., *J. Am. Chem. Soc.* **30**, 215 (1908); see also U. S. Geol. Survey, *Bull.* **700**, 37 (1919).
- FRANKFORTER, G. B., WALKER, G. W., and WILHOIT, A. D., *J. Am. Chem. Soc.* **31**, 35 (1909). Colorimetric determination of dissolved oxygen in water. Give a detailed description of their apparatus and a figure.
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- GÜNTHER, T., *Chem.-Ztg.* **33**, 318 (1909).
- FUEAT, E. and SCHLESINGER, E., German Patent No. 235,541 (1910). A bicol-  
orimeter.
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investigation. May also be used at room temperature.

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- MECKLENBURG, A. W. and VALENTINER, S., *Z. Instrumentenk.* **34**, 209 (1914); *Kolloid Z.* **14**, 172 (1914). Tyndallmeter.
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- SPINDLER, F. G., U. S. Pat. 1,353,500, Sept. 21, 1920; *J. Soc. Chem. Ind.* **39**, 769A (1920).
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- HAAN, J. DE, *Nederland. Tijdschr. Geneeskunde* **66**, 1076 (1922); *C. A.* **16**, 2699 (1922). A simple colorimeter for clinical use.
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- STANFORD, R. V., *Biochem. J.* **17**, 839 (1923); *J. Chem. Soc.* **126**, ii, 198 (1924); *C. A.* **18**, 769 (1924). Improvements in colorimetry. Describes improvements in his inconvenient colorimeter reported in *Z. physiol. Chem.* **87**, 159 (1913), as well as improvement in the source of light employed.
- KING, W. J., *Ind. Eng. Chem.* **15**, 351 (1923). Essentially the Campbell and Hurley type, but with certain improvements for use in determining Mo. Gives a diagrammatic drawing of the apparatus.
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- HIBBARD, P. L., *Ind. Eng. Chem.* **16**, 804 (1924). A modified Campbell-Hurley colorimeter which affords a rapid means for the determination of any ion which will give a colorless granular precipitate by measurement of turbidity. Must be calibrated for each suspension. Accuracy 5-10 per cent. Methods for Ca, Mg, and SO<sub>4</sub> given.
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- THRO, W. C., *J. Lab. Clin. Med.* **10**, 491 (1924-25). Modifications of the Sahli hemoglobinometer.
- LEBERMANN, F., *Münch. med. Wochschr.* **72**, 982; *Chem. Zentr.* **1925**, II, 1199; *C. A.* **20**, 3470 (1926). The utility of the Buerker colorimeter, with special reference to the determination of hemoglobin.
- SCOTT, W. W., *Standard Methods of Chemical Analysis*, 4 ed., D. Van Nostrand Co., New York, 1925. Describes (with figures) several types of colorimeters on pp. 164b, 283, 545, 546, and 548.
- DOLD, H., *Chem.-Ztg.* **49**, 842 (1925); *C. A.* **20**, 1 (1926). A description of a tubidometer sold by F. and M. Lautenschläger, Frankfurt a. M.
- OLSZEWSKI, W., *Chem.-Ztg.* **50**, 694 (1926); *C. A.* **21**, 197 (1927). The Olszewski-Rosemüller turbidimeter and colorimeter for water investigation. De-

- scribes a half-shadow photometer in which the color glasses developed by the U. S. Geological Survey are used for the color determinations.
- REIMANN, S. P., *Proc. Soc. Exptl. Biol. Med.* **23**, 520 (1926); *C. A.* **21**, 1034 (1927). The photoelectric cell as a colorimeter. Methods are described for using the photoelectric cell in colorimetric and nephelometric determinations. The advantages are extreme accuracy and simplicity.
- BERNOULLI, A. L., *Helv. Chim. Acta.* **9**, 827 (1926); *C. A.* **21**, 30 (1927). The sliding-gage colorimeter and the determination of minute quantities of ammonia, nitrite, lead, and iron. By means of this instrument it is possible to det. 0.002 mg. of Fe dissolved in 0.1 cc. with an accuracy of 0.4 of 1 per cent of the total amt. present.
- FREUND, H., *Chem.-Ztg.* **50**, 194 (1926). A new colorimeter. Manufacturer's announcement.
- KLEINMANN, H., *Biochem. Z.* **179**, 276 (1926). Ein neues Kolorimeter für kleine Flüssigkeitsmengen (Mikrokolorimeter).
- BADER, J. P., U. S. Pat. 1,629,609, May 24, 1927; *C. A.* **21**, 2202 (1927). A colorimeter adapted for tests of hydrocarbon oils.
- YOE, J. H., *Ind. Eng. Chem.* **19**, 1131 (1927). A colorimeter for precise matching solutions in Nessler tubes. Similar to the Kennicott-Campbell-Hurley colorimeter, a series of Nessler tubes and a rack replacing the stand carrying the two comparison cylinders and reservoir tube in the latter apparatus.

#### Colorimeter Curve.

- WRIGHT, S. L., Jr., *J. Biol. Chem.* **71**, 209 (1926-27). Note on colorimeter correction curves.

#### Colorimeter Lamp.

- DAY, H. D., *Proc. Physiol. Soc., J. Physiol.* **62**, XXXI (1927); *C. A.* **21**, 2144 (1927). A simple colorimeter lamp.

#### Colorimetry.

- CAMERON, C. A., *Chem. News* **34**, 77 (1876). On the estimation of color in water.
- BOTTOMLEY, J., *Chem. News* **38**, 191 (1878); *J. Chem. Soc.* **36**, 77 (1879). General theory and a colorimeter similar to E. J. Mills'.
- BOTTOMLEY, J., *Chem. News* **39**, 276 (1879). General theory.
- HAZEN, A., *Am. Chem. J.* **14**, 300 (1892); *J. Chem. Soc.* **62**, 1527 (1892). A new color standard for natural waters. Uses Pt and Co chlorides.
- LOVIBOND, J. W., *J. Soc. Chem. Ind.* **13**, 308 (1894).
- BARBET, E., *Bull. assocn. chim. sucr. dist.* **25**, 40; *C. A.* **2**, 41 (1908). Standard unity for colorimetry. Proposes the following definition of the "coloric." The coloration produced by a solution of 10 mm., containing 0.1 g. of iodine to the liter.
- SCHREINER, O., *J. Am. Chem. Soc.* **27**, 1192 (1905); also *Bull. No. 31*, U. S. Dept. Agr., Bur. of Soils (1906). A simple colorimeter for general use, with remarks on colorimetric methods and apparatus. Introductory remarks on the various applications of colorimetry. Well-known and also new meth-

- ods for determining small quantities of  $\text{NH}_4$ , K, Mg, Mn, Fe, Ti,  $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{PO}_4$ ,  $\text{SiO}_2$ ,  $\text{SO}_4$ , S,  $\text{CaCl}_2$ ,  $\text{CaCO}_3$ , and  $\text{Ca}(\text{HCO}_3)_2$ .
- KNAPP, A. W., J. Soc. Chem. Ind. **29**, 1343 (1910); C. A. **5**, 822 (1911). The interpretation of color values obtained by Lovibond's tintometer. Also contains a discussion by Liversedge on the application of the tintometer to the examination of water.
- MÖLLER, H. J., Ber. deut. pharm. Ges. **20**, 358; Pharm. Zentralhalle **51**, 1059; C. A. **5**, 1000 (1911). International color determinations. Gives a summary of the various schemes for color standards and describes in more detail the "code of colors" of P. Klincksieck, Bull. soc. bot. France. **53**, (1906) and Bull. soc. mycol. France. **22** (1906).
- LOVIBOND, J. W., Pottery Gaz. **35**, 1269; C. A. **5**, 227 (1911). Describes the principles and application of the Lovibond tintometer.
- FALK, K. G. and NOYES, H. M., J. Biol. Chem. **42**, 109 (1920); C. A. **14**, 2355 (1920).
- SNELL, F. D., Colorimetric Analysis, D. Van Nostrand Co., New York, **1921**, 150 pp. Gives one or more methods for each of the following substances: Fe, Cu, C, Pb, Bi, As, Al, Cr, Ni, Co, Mn, Zn, K, Mg, Au, Ti, V, W, F, Cl,  $\text{ClO}_4$ ,  $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{NH}_3$ ,  $\text{PO}_4$ ,  $\text{SiO}_2$ , B, O,  $\text{H}_2\text{O}_2$ , S,  $\text{H}_2\text{S}$ ,  $\text{SeO}_2$ , CN, salicylic acid, and color of water, oils, and dyes.
- FLEURY, P., Bull. soc. chim. biol. **4**, 223 (1922); C. A. **16**, 3598 (1922); J. Chem. Soc. **122**, ii, 518 (1922).
- DOSNE, P., Bull. soc. ind. Mulhouse **88**, 73 (1922); J. Chem. Soc. **122**, ii, 518 (1922); J. Soc. Chem. Ind. **41**, 485A (1922). A bright beam of light is directed upwards through a column of a solution of known strength of the colored substance under examination and the height of the column necessary for the complete absorption of the transmitted light, as observed through a spectroscope, is determined. The coloring power of a substance is directly proportional to the height of the column and to the strength of the solution; hence, various coloring substances can be compared when dissolved in water, alcohol, and other solvents. Method useful but not new.
- WULFF, P., German Patent No. 405,091 (1923). Aids to colorimetric determination of dissolved chemical compounds. Uses gels.
- YOE, J. H., J. Lab. Clin. Med. **13**, 139 (1927). A general discussion on colorimetry. The following topics are treated: (1) methods of matching color, (2) requirements, (3) accuracy, (4) speed, (5) limits of application, and (6) errors.

### Columbium.

- LEVY, L., Compt. rend. **103**, 1074, 1195; see also J. Anal. Chem. **1**, 201 (1887). Colored reactions of the rare mineral acids. Titanic, niobic, tantalalic, stannic, arsenic, and vanadic acids, and bismuth oxide. Reagents used were either phenols or allied substances.
- MEIMBERG, E., Z. angew. Chem. **26**, 83 (1913). Makes use of the fact that columbates, especially the fluoride compounds, are reduced by tin and HCl to colored compounds, whereas the tantalates are not affected. 0.1 per cent of columbium can be estimated with an error not exceeding 5 per cent.

MEIMBERG, E. and WINZER, P., *Z. angew. Chem.* **26**, I, 157 (1913). Separate Ta from columbium by precipitation of former with KCl and HF.

### Copper.

- KEATES, Z. *anal. Chem.* **5**, 424 (1866). Keates said to be the first person to determine copper colorimetrically. This was done in 1830. Cited in *Z. anal. Chem.* **5**, 424 (1866).
- HEINE, *Bergwerksfreund* **1**, 33 and **17**, 405; reference made in *Z. anal. Chem.* **5**, 424 (1866). Work done a few years later than that of Keates in 1830.
- JACQUELAIN, J. *prakt. Chem.* **46**, 174 (1849).
- HUBERT, *Berg. u. hüttenm. Ztng.* **1849**, p. 677 and **1851**, p. 804. See also *Schmeltzer u. Leobener Jahrb.* **14**, 187 (1865) and *Bergwfrnd.* **17**, 405; references given in *Z. anal. Chem.* **5**, 424 (1866).
- MÜLLER, A., *J. prakt. Chem.* **60**, 474 (1853) and **66**, 193 (1855); *Z. anal. Chem.* **5**, 423 (1866).
- EGGERTZ, *Berg. u. hüttenm. Ztng.* **1862**, p. 218; reference given in *Z. anal. Chem.* **5**, 424 (1866). Colorimetric method for determining small amounts of copper in iron ores, iron and steel.
- DEHMS, F., *Z. anal. Chem.* **3**, 218 (1864). Uses  $\text{NH}_4\text{OH}$ .
- WAGMEISTER, *Oesterr. Zeit.* **1865**, p. 270; reference given in *Z. anal. Chem.* **5**, 424 (1866).
- MÜLLER, A., *J. prakt. Chem.* **99**, 337 (1866); *Z. anal. Chem.* **6**, 252 (1867).
- BISCHOF, G., Jr., *Dingler's polytech. J.* **184**, 433; *Z. anal. Chem.* **6**, 459 (1867). Uses  $\text{NH}_4\text{OH}$ . Describes his colorimeter.
- BLUNT, T. P., *Chem. News* **32**, 3 (1875). Estimation of traces of Cu in red lead. Uses  $\text{K}_4\text{Fe}(\text{CN})_6$ .
- BERGERON and L'HÔTE, L., *Compt. rend.* **80**, 268 (1875). Estimation of very minute traces of copper in the human body. Use  $\text{NH}_4\text{OH}$  and  $\text{K}_4\text{Fe}(\text{CN})_6$  methods.
- CARNELLEY, T., *Chem. News* **32**, 308 (1875); *J. Chem. Soc.* **30**, 751 (1876). Uses  $\text{K}_4\text{Fe}(\text{CN})_6$ . Will detect 1 part of Cu in 2,500,000 parts of water.
- BLUNT, T. P., *Chem. News* **33**, 7 (1876). Claims priority over Carnelley [*Chem. News* **32**, 308 (1875)] in using the  $\text{K}_4\text{Fe}(\text{CN})_6$  method, and discusses colorimetry. Suggests that judgment by turbidity may be provisionally called "nephelometry."
- MUIR, M. M. P., *Chem. News* **33**, 11 (1876). Uses  $\text{H}_2\text{S}$ .
- BAYLEY, T., *Chem. News* **41**, 170 (1880); *Z. anal. Chem.* **19**, 470 (1880). On the reflection from copper, and on the colorimetric estimation of copper by means of the reflection cuprimeter.
- WAGNER, A., *Z. anal. Chem.* **20**, 349 (1881); *Chem. News* **45**, 35 (1882). On the delicacy of the  $\text{K}_4\text{Fe}(\text{CN})_6$ , KCNS, and tannic tests for Fe and the  $\text{K}_4\text{Fe}(\text{CN})_6$ ,  $\text{NH}_4\text{OH}$  and K xanthogenate tests for Cu.
- NESSLER, J. and BARTH, M., *Z. anal. Chem.* **22**, 37 (1883). Use  $\text{K}_4\text{Fe}(\text{CN})_6$ .
- COOPER, A. J., *J. Soc. Chem. Ind.* **5**, 84 (1886). Note on the detection of metals in drinking water. Gives a table showing the delicacy of the following tests:  $\text{K}_4\text{Fe}(\text{CN})_6$ ,  $\text{NH}_4\text{OH}$ , and  $\text{H}_2\text{S}$  tests for Cu;  $(\text{NH}_4)_2\text{S}$  test for Zn;  $\text{H}_2\text{S}$  test for As;  $\text{K}_2\text{CrO}_4$  and  $\text{H}_2\text{S}$  tests for Pb.

- ZIEGLER, A., Dingler's polytech. J. **285**, 142 (1892).
- BUDDEN, E. R. and HARDY, H., Analyst, **19**, 169; J. Chem. Soc. **66**, ii, 481 (1894). Colorimetric estimation of minute quantities of lead, copper, tin, and iron. In determining traces of metals by the hydrogen sulfide method it is necessary to follow strictly the same order in the addition of the reagents and, as closely as possible, to use always the same quantity of reagents, both in the experiment and in the preparation of the standard for comparison.
- BUDDEN, E. R. and HARDY, H., Analyst **21**, 12 (1896). Find the colorimetric methods preferable to electrolytic methods for testing beverages for metals when several are present. Metals tested: Cu, Pb, Hg.
- HEATH, G. L., J. Am. Chem. Soc. **19**, 24 (1897). Improvements in the colorimetric test for copper. Uses  $\text{NH}_4\text{OH}$ . Gives precautions to be taken.
- BODMER, R. and MOOR, C. G., Analyst **22**, 141 (1897). On copper in peas. Uses the following colorimetric methods: (1)  $\text{H}_2\text{S}$ , (2)  $\text{NH}_4\text{OH}$ , (3) ferrocyanide.
- LUCAS, M., Bull. soc. chim. **19**, 815 (1898); J. Chem. Soc. **76**, ii, 522 (1899). Uses  $\text{K}_4\text{Fe}(\text{CN})_6$ .
- BACH, A., Compt. rend. **128**, 363 (1899); cf. Thomas and Carpentier, *ibid.* **173**, 1084 (1921). Formaldoxime as a reagent for detecting very small quantities of copper. Sensitive to approximately 1 part of Cu in  $3\frac{1}{2}$  million parts of water.
- PHELPS, E. B., J. Am. Chem. Soc. **28**, 368 (1906). Estimation of small quantities of copper in (drinking) waters. Uses alkaline  $\text{K}_2\text{S}$ . Accurate to 0.1 part per million.
- BRADLEY, H. C., Am. J. Sci. [4], **22**, 326 (1906); C. A. **1**, 150 (1907). Logwood hematoxylin produces a dark blue color with copper. Reaction extraordinarily delicate. 0.061 per cent copper gives a blue color. One thousand times more delicate than the ferrocyanide test. Conditions for optimum reaction and its nature not yet worked out.
- MILBAUER, J., Z. anal. Chem. **46**, 656 (1907); C. A. **2**, 972 (1908). Colorimetric comparison of solutions of copper and nickel salts. Uses  $\text{NH}_4\text{OH}$ .
- MILBAUER, J. and STANĚK, V., Z. anal. Chem. **46**, 644 (1907); C. A. **2**, 971 (1908). A study of the effect of the presence of  $\text{NH}_3$ ,  $\text{NH}_4\text{Cl}$  and  $(\text{NH}_4)_2\text{CO}_3$  in various amounts upon the intensity and shade of the blue color of the complex copper-ammonia ions.
- UHLINHUTH, R., Chem.-Ztg. **34**, 887 (1910); Analyst **35**, 453 (1910). A new reaction of copper. Uses an alkaline solution of 1.2-diamino-anthra-quinone-3-sulphonic acid. Intense blue color obtained. Will detect 0.00019 mg. Cu per cubic centimeter.
- AUSTIN, A., Mining World **33**, 753; C. A. **5**, 1038 (1911). Uses  $\text{NH}_4\text{OH}$ .
- SERGER, H., Chem.-Ztg. **35**, 935 (1911); C. A. **5**, 3704 (1911). Colorimetric determination of copper in preserves. Uses  $\text{NH}_4\text{OH}$ .
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### Creatinine.

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- $$S_4O_6^{--} + CN^- + 2NH_3 \rightarrow CNS^- + S_2O_3^{--} + SO_3^{--} + 2NH_4^+$$
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of hydrogen by NaOH and  $\text{Na}_2\text{S}_2\text{O}_4$  and the solutions matched against standardized blue glasses in a Kennicott-Campbell-Hurley colorimeter.

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### Errors.

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Errors resulting from varied quantities of NaOH in Folin's creatinine estimation.

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**Ethyl Nitrite.**

COWLEY, R. C. and CATFORD, J. P., *Pharm. J.* **63**, 471 (1899). Use *m*-phenylene-diamine.

**Fat.**

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**Fluorine.**

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STEIGER, G., *J. Am. Chem. Soc.* **30**, 219 (1908). Makes use of the fact that fluorides bleach the coloration produced by  $\text{H}_2\text{O}_2$  and Ti. Since this is not a linear function, reference is made to a curve.

MERWIN, H. E., *Am. J. Sci.* **28**, 119 (1909); *C. A.* **3**, 2919 (1909).  $\text{H}_2\text{O}_2$  used. Color produced by Ti, and the bleaching effect of F, used to estimate Ti and F. respectively. Effects of free acid, alkali salts, and temperature given.

GAUTIER, A. and CLAUSMANN, P., *Ann. fals.* **5**, 329; *Compt. rend.* **154**, 1469 (1912); *C. A.* **6**, 2900 (1912). Method for the detection and determination of fluorine in waters, minerals, tissues, and foods. F precipitated as  $\text{PbF}_2$  and Pb estimated colorimetrically, then F calculated.

GAUTIER, A. and CLAUSMANN, P., *Compt. rend.* **154**, 1670 (1912); *C. A.* **6**, 2727 (1912); *J. Chem. Soc.* **102**, ii, 805 (1912); *J. Soc. Chem. Ind.* **31**, 703 (1912); cf. *Compt. rend.* **154**, 1469 and 1753. Detection and colorimetric determination of very small quantities of fluorine. A long procedure. F liberated by  $\text{H}_2\text{SO}_4$  as HF and  $\text{H}_2\text{SiF}_6$ , and dissolved in fused KOH. F precipitated in conjunction with  $\text{BaSO}_4$ . Again treat with  $\text{H}_2\text{SO}_4$  using lead glass instead of fused KOH.  $\text{PbF}_2$  formed. Pb precipitated as colloidal PbS by  $\text{H}_2\text{S}$ . A little gelatin is added before precipitation.

SNELL, F. D., *Colorimetric Analysis*, p. 102, D. Van Nostrand Co., New York, 1921. Fluorine by estimation of its bleaching action on an oxidized titanium solution.

SCOTT, W. W., *Standard Methods of Chemical Analysis*, 4 ed., pp. 220-222, D. Van Nostrand Co., New York, 1925. Uses method of Steiger [*J. Am. Chem. Soc.* **30**, 219 (1908)] and Merwin [*Am. J. Sci.* [4], **28**, 119 (1909)].

**Furfural.**

DE CHALMOT, G., *Am. Chem. J.* **15**, 25 (1893). Uses HAc, and 1 per cent solution of aniline in 95 per cent alcohol. Red color produced.

TOLMAN, L. M., *J. Am. Chem. Soc.* **28**, 1629 (1906). Uses aniline (colorless) and HCl.

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YOUNGBURG, G. E., and PUCHER, G. W., *J. Biol. Chem.* **61**, 741 (1924). Use aniline and HAc. Red color formed. Detects 0.00001 per cent. Quantitative for 0.00004 per cent.

**Gland.**

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**Glucose.**

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- NEITZEL, E., *Chem.-Ztg.* **18**, I, 290 (1894); *Z. Rübenzucker-ind.* **1894**, 221. Uses a modification of Molisch reagent (*Monatsh.* **7**, 198).
- RUINI, G., *Gazz. chim. ital.* **31**, 445 (1901).
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- AUTENRIETH, W. and FUNK, A., *Münch. med. Wochschr.* **59**, 689; *C. A.* **6**, 2088 (1912). The colorimetric method for determining glucose and indican in urine, as also the iron in the blood.
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- TAYLOR, A. E. and HULTON, F., *J. Biol. Chem.* **22**, 63 (1915). On the estimation of non-protein nitrogen and glucose in finger blood. A modification of the Folin method.
- HENDRIX, B. M. and SWEET, J. E., *J. Biol. Chem.* **32**, 299 (1917). Glucose by method of Lewis and Benedict, *J. Biol. Chem.* **20**, 61 (1915).
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- RICHARDSON, H. B. and MASON, E. H., *J. Biol. Chem.* **27**, 587 (1923). Glucose by Benedict's method.
- UPDEGRAFF, H. and LEWIS, H. B., *J. Biol. Chem.* **61**, 633 (1924). A quantitative study of some organic constituents of the saliva. Nitrogen and glucose by Folin-Wu method. Uric acid by Benedict's method.
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- HAWK, P. B. and BERGEIM, O., *Practical Physiological Chemistry*, 9 ed., P. Blakiston's Son and Co., Philadelphia, **1926**. Determination of glucose in blood, pp. 381-388; in urine, p. 751.

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#### Glutathione.

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#### Glycogen.

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#### Gold.

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- CARNOT, A., *Compt. rend.* **97**, 105, 169 (1883); *J. Chem. Soc.* **46**, 115 (1884). Uses  $\text{H}_3\text{AsO}_4$ ,  $\text{FeCl}_3$ , and  $\text{HCl}$  and adds  $\text{Zn}$ .
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- CARNOT, *Berg- und Hüttenmann Z.* **55**, 215 (1896); *J. Soc. Chem. Ind.* **15**, 674 (1896). Uses  $\text{H}_3\text{AsO}_4$ ,  $\text{FeCl}_3$ ,  $\text{HCl}$  and  $\text{Zn}$ .
- MOIR, *J. Chem. Met. Mining Soc. South Africa*, Sept., **1903**. Method is based upon formation of purple of Cassius.
- CASSEL, H. R., *Eng. Mining J.* **76**, 661 (1903). Gold by decomposition of the cyanide by (1)  $\text{KBrO}_3$  and conc.  $\text{H}_2\text{SO}_4$ , (2)  $\text{KBr}$ ,  $\text{Na}_2\text{O}_2$  or  $\text{K}_2\text{O}_2$ , and  $\text{H}_2\text{SO}_4$ , or (3) successive additions of strong  $\text{NH}_4\text{OH}$  and conc.  $\text{H}_2\text{SO}_4$ .  $\text{SnCl}_2$  finally added.
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- of gold chloride. Determinations made with solutions containing 0.00004 g. to 0.0008 g. Au. Maximum error 0.00006 g.
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- BETTEL, W., Mining Eng. World **33**, 102; C. A. **5**, 651 (1911). Estimating gold in cyanide solution. Uses a Zn-Cu couple to precipitate the Au. The couple is prepared by adding to the Au solution a measured quantity of strong cyanide solution containing some CuCN, and Zn. The extra cyanide keeps ferrocyanide, etc., in solution. The solution is then heated, with stirring to keep the Zn-Cu couple in suspension, till the Au is precipitated and the process completed in about the usual way.
- BETTEL, W. Mining Eng. World **35**, 987; C. A. **6**, 842 (1912); cf. C. A. **5**, 651. Colorimetric estimation of gold in cyanide solutions.
- SIEMSEN, J. A., Chem.-Ztg. **36**, 934 (1912). Uses metaphenylenediamine.
- BRODIGAN, C. B., Met. Chem. Eng. **12**, 460 (1914); C. A. **8**, 2990 (1914). Colorimetric estimation of gold in cyanide solutions. Uses NaCN,  $\text{Pb}(\text{NO}_3)_2$ , Zn, and  $\text{SnCl}_2$ . Gets purple color.
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- SNELL, F. D., *ibid.*, p. 91, 1921. Determination of gold by Doring's method.
- SNELL, F. D., *ibid.*, p. 92, 1921. Determination of gold by Rose's method.
- SNELL, F. D., *ibid.*, p. 93, 1921. Determination of gold by Cassel's method.
- SNELL, F. D., *ibid.*, p. 93, 1921. Determination of gold by decomposition of the cyanide by KBr and  $\text{Na}_2\text{O}_2$ .
- SNELL, F. D., *ibid.*, p. 94, 1921. Determination of gold by decomposition of the cyanide by ammonia.
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- MULLER, J. A. and FOIX, A., Bull. soc. chim. [iv], **33**, 717 (1922); J. Chem. Soc. **122**, ii, 662 (1922); Analyst **47**, 452 (1922); J. Soc. Chem. Ind. **41**, 731A (1922). The estimation of small quantities of gold as colloidal gold by the colorimetric method.
- SCOTT, W. W., Standard Methods of Chemical Analysis, 4 ed., p. 233, D. Van Nostrand Co., New York, 1925. Uses (1) Prister's method, (2) Cassel's method, (3) Moir's method.

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- HOPPE-SEYLER, F., Z. physiol. Chem. **16**, 505 (1892); J. Chem. Soc. **62**, 1264 (1892).
- ZANGEMEISTER, W., Z. Biol. **33**, 72 (1896).
- HALDANE, J., J. Physiol. **26**, 497 (1900-01).
- SAMNELY, F., Münch. med. Wochschr. **57**, 1545; C. A. **4**, 2656 (1910). The application of the Autenrieth-Koenigsberger colorimeter as a Kerno-globinometer. Describes the technique in detail for estimating the hemoglobin content of the blood. Gives diagrams showing the per cent of hemoglobin from 10-100 with the corresponding readings.
- PALMER, W. W., J. Biol. Chem. **33**, 119 (1918). Uses CO. Accuracy 1 per cent.
- BERCZELLER, L., Biochem. Z. **87**, 23 (1918); J. Chem. Soc. **114**, ii, 340; C. A. **13**, 28 (1919). The colorimetric estimation of hemoglobin as acid hematin.
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- NEWCOMER, H. S., J. Biol. Chem. **37**, 465 (1919).
- COHEN, B. and SMITH, A. H., J. Biol. Chem. **39**, 489 (1919). Use HCl. Accuracy to 1 per cent or less.
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- ROBSCHETT, F. S., J. Biol. Chem. **41**, 209 (1920). A comparative study of hemoglobin determination by various methods.
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CULLEN, G. E., KEELER, H. R. and ROBINSON, H. W., *J. Biol. Chem.* **66**, 301 (1925).

LEBERMANN, F., *Münch. med. Wochschr.* **72**, 982; *Chem. Zentr.* **1925**, II, 1199; *C. A.* **20**, 3470 (1926). The utility of the Buerker colorimeter, with special reference to the determination of hemoglobin.

KENNEDY, R. P., *Am. J. Physiol.* **78**, 56 (1926); *C. A.* **20**, 3475 (1926). The use of light filters in colorimetry with a method for the estimation of hemoglobin.

#### Heroine.

MILLER, R., *Am. J. Pharm.* **87**, 248 (1915); *J. Chem. Soc.* **108**, ii, 602 (1915). Estimation of small quantities of heroine. Uses  $H_2SO_4$  and  $HCHO$ . Yellow to cherry-red color develops in 10 minutes.

MULLER, Riv. farm.; *Giorn. farm. chim.* **66**, 227 (1917); *C. A.* **12**, 1333 (1918). Rapid method for determining small amounts of heroine. Uses dilute  $H_2SO_4$  and  $HCHO$ .

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COLLINS, R. J. and HANZLIK, P. J., *J. Biol. Chem.* **25**, 231 (1916). Use Congo red and methyl orange.

#### Histamine.

KOESSLER, K. K. and HANKE, M. T., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.* **41**, 1 (1920). Quantitative colorimetric determination of histidine and histamine in proteins and protein-containing matter. Based on method described in *J. Biol. Chem.* **10**, 15 (1911-12).

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EDEN, T., J. Agr. Sci. **14**, 469 (1924); J. Chem. Soc. **126**, ii, 796 (1924); Analyst **49**, 491 (1924). Colorimetric determination of humic matter in mineral soils.

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KASTLE, J. H. and AMOSS, H. L., Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **3**, xi (1907). A new reagent for the recognition and estimation of free hydrochloric acid in gastric contents. Coloring matter of red cabbage.

DELORT, M. and ROEKE, Compt. rend. soc. biol. **81**, 646 (1918). Colorimetric scale for the quick evaluation of the free HCl of a given solution.

SHOHL, A. T. and KING, J. H., Johns Hopkins Hosp. Bull. **31**, 158 (1920).

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DENIS, W. and MINOT, A. S., J. Biol. Chem. **37**, 245 (1919). H-ion by method of Henderson and Palmer.

KARR, W. G., with the cooperation of E. TOLSTOI, J. Biol. Chem. **45**, 289 (1920-21). Comparative metabolism of proteins of unlike composition. H-ion by Sørensen colorimeter method.

FISKE, C. H., J. Biol. Chem. **49**, 163 (1921).

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- MCQUARRIE, I. and SHOHL, A. T., *J. Biol. Chem.* **66**, 367 (1925). A colorimetric method for the determination of the  $pH$  of cerebrospinal fluid. Use phenol red.
- AUSTIN, J. H., STADIE, W. C. and ROBINSON, H. W., *J. Biol. Chem.* **66**, 505 (1925). The relation between colorimetric reading and true  $pH$  of serum or plasma. Show that there is considerable variation in the difference between colorimetric and true  $pH$  and outline method for correction.
- LENNOX, W. G. with the assistance of M. O'CONNOR, *J. Biol. Chem.* **66**, 521 (1925). A study of the retention of uric acid during fasting.  $pH$  by Cullen's method.
- STADIE, W. C., AUSTIN, J. H. and ROBINSON, H. W., *J. Biol. Chem.* **66**, 901 (1925). The effect of temperature on the acid-base-protein equilibrium and its influence on the  $CO_2$  absorption curve of whole blood, true and separated serum.  $pH$  by Cullen's method.
- HOLWERDA, B. J., *Verslag. Landw. Onderzoek. Rijkslandbouwpfrolfsta.* No. 30, 220 (1925); *C. A.* **20**, 245 (1926). The colorimetric determination of the acidity of cheese and whey.
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- C. A. **20**, 3468 (1926). The use of the bicolorimeter for the estimation of the hydrogen ion concentration of urine.
- SHAXBY, J. H. and JONES, O. M., *Proc. Physiol. Soc., J. Physiol.* **61**, xxvi (1926); C. A. **20**, 3475 (1926). The colorimetric determination of hydrogen ion concentration.
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- BENNETT, MARY A., *J. Biol. Chem.* **69**, 697 (1926). A note on Cullen's colorimetric method for the determination of the pH of blood plasma.
- JANKE, A. and KROPACSY, S., *Biochem. Z.* **174**, 120 (1926); C. A. **21**, 872 (1927).
- MCCLENDON, J. F., RUSSELL, S. and TRACY, E., *J. Biol. Chem.* **70**, 705 (1926). Use a modified Duboscq colorimeter and ortho-chrom-T or para-nitrophenol as indicators in the determination of the pH in blood plasma.
- PARSONS, L. B. and DOUGLAS, W. F., *J. Bact.* **12**, 263 (1926); C. A. **21**, 112 (1927). The influence of sodium chloride on the colorimetric determination of pH.
- SHARP, P. F. and MCINERNEY, T. J., *J. Biol. Chem.* **70**, 729 (1926). pH of milk, whey, and cream.
- SCHLEGEL, J. W. and STEUBER, A. H., *Ind. Eng. Chem.* **19**, 631 (1927); C. A. **21**, 2109 (1927). Some sources of error in the colorimetric determination of pH values.
- ROSÉE and MÖRGENSTERN, v., *Chem.-Ztg.* **51**, 302 (1927); C. A. **21**, 2235 (1927). A simplified modification of the Tödt colorimetric method for determining the hydrogen-ion concentration in small quantities of highly colored or turbid solutions.
- TAYLOR, W. A., "The A B C of Hydrogen Ion Control" (pamphlet), 4 ed., La-Motte Chemical Products Co., Baltimore, Md., 1928.

### Hydrogen Peroxide.

- SCHÖNN, Z. anal. Chem. **9**, 41, 330 (1870). First to observe the yellow color produced by the action of  $H_2O_2$  on molybdic and titanous acids.
- SCHÖNE, E., Z. anal. Chem. **18**, 154 (1879).
- JACKSON, E., *Chem. News* **47**, 157 (1883).  $H_2O_2$  by  $TiO_2$  in  $HCl$  or  $H_2SO_4$ . Suggests that the test may be made quantitative.
- CRISMER, L., *Gaz. med. Liège* **7**, 77 (1888). Discovered the yellow color produced by the action of  $H_2O_2$  on a molybdate in acid solution. Did not know of Schönn's discovery (1870) of the same reaction.
- DENIGÈS, G., *Compt. rend.* **110**, 1007 (1890); *J. Chem. Soc.* **58**, 1185 (1890). Used solution of ammonium molybdate in concentrated  $H_2SO_4$ . Blue solution obtained.
- CRISMER, L., *Bull. soc. chim. [3]*, **6**, 22 (1891); *J. Chem. Soc.* **62**, 381 (1892).
- RICHARDSON, A., *J. Chem. Soc.* **63**, 1110 (1893). Uses  $TiO_2$  in  $H_2SO_4$ .
- PLANÈS, P., *J. pharm. chim. [vi]*, **20**, 538 (1904); *J. Chem. Soc.* **88**, ii, 199 (1905); *J. Soc. Chem. Ind.* **24**, 45 (1905). Method based upon the following equa-

- tion:  $2KI + H_2O_2 + H_2SO_4 \rightarrow K_2SO_4 + 2H_2O + I_2$ . Color matched against a 0.1N iodine solution.
- AMBERG, S., *J. Biol. Chem.* **1**, 219 (1905-06). A method for the determination of hydrogen peroxide in milk. Uses titanium sulfate in  $H_2SO_4$  solution. Salicylic acid gives a similar color and hence must be absent.
- ROGAI, A., *Staz. sper. agrar. ital.* **67**, 569 (1914); *Ann. chim. applicata* **2**, 341; *C. A.* **9**, 772 (1914). A very sensitive test for hydrogen peroxide. Uses  $FeSO_4$ , KCNS, and  $Et_2O$ . Only small quantity of  $H_2O_2$  can be determined accurately.
- QUARTAROLI, A., *Gazz. chim. ital.* **48**, I, 102 (1918). *C. A.* **13**, 1684 (1919); *Chem. Zentr.* **93**, II, 728 (1922); *Z. anal. Chem.* **62**, 469 (1923). Estimation of minute quantities of nitrites and hydrogen peroxide in presence of each other. Reagent—about 6 per cent KCNS solution to which 0.5 g. crystalline  $FeSO_4$  in 2 cc. pure colorless  $HNO_3$  + 2 cc.  $H_2O$  has been added, and the mixture agitated, 2 cc. of the reagent are added to 25 cc. of the test solutions. Special tests are described for  $H_2O_2$  and  $HNO_2$  when both are present.
- HORST, F. W., *Chem.-Ztg.* **45**, 572 (1921); *J. Chem. Soc.* **120**, ii, 461 (1921); *J. Soc. Chem. Ind.* **40**, 507 (1921). Detection and estimation of traces of hydrogen-peroxide.  $H_2O_2$  reduced by  $FeSO_4$  and the  $Fe_2(SO_4)_3$  determined by  $NH_4CNS$ .
- SNELL, F. D., *Colorimetric Analysis*, p. 133, D. Van Nostrand Co., New York, 1921. Determination of hydrogen peroxide by its oxidizing action on ferrous iron.
- ISAACS, M. L., *J. Am. Chem. Soc.* **44**, 1662 (1922); *C. A.* **16**, 3282 (1922); *Analyst* **47**, 490 (1922); *J. Soc. Chem. Ind.* **41**, 751A (1922). Yellow color produced by  $H_2O_2$  and  $MoO_3$  in citric acid solution.

### Hydrogen Sulfide.

- FISCHER, E., *Ber.* **16**, 2234 (1883). Calls attention to the possibility of using *p*-amino-dimethylaniline as a reagent for the detection of  $H_2S$  in exceedingly small quantities. This compound and  $H_2S$  in the presence of  $HCl$  and an oxidizing agent ( $FeCl_3$ ) react to form methylene blue. This is generally known as Lauth's reaction [cf. Almy, *J. Am. Chem. Soc.* **47**, 1382 (1925)].
- HUYSE, A. C., *Chem. Zentr.* **1894**, ii, 108; from *Nederl. Tydschr. Pharm.* **6**, 167; *J. Chem. Soc.*, 68, ii, 289 (1895). Method based upon formation of methylene blue. Uses *p*-amino-dimethylaniline hydrochloride and  $FeCl_3$ .
- WINKLER, L. W., *Z. anal. Chem.* **40**, 772 (1901); *Analyst* **27**, 104 (1902). The determination of small quantities of hydrogen sulfide in natural waters. Uses Na K tartrate,  $NaOH$ , and  $PbAc_2$ .
- AUTENRIETH, W. and FUNK, A., *Z. anal. Chem.* **52**, 137 (1913); *C. A.* **7**, 3627 (1913). Colorimetric methods for water analysis by the use of the Autenrieth-Koenigsberger colorimeter. Details are given for the estimation of  $NH_3$ ,  $HNO_2$ ,  $HNO_3$ ,  $Fe$ ,  $Pb$ , and  $H_2S$ .
- WINKLER, L. W., *Z. anal. Chem.* **52**, 641 (1913); *J. Soc. Chem. Ind.* **32**, 957 (1913). Determination of hydrogen sulfide in potable water. Uses  $PbAc_2$ . Colloidal suspension of  $PbS$  is formed.



- MECKLENBURG, W. and ROSENKRÄNZER, F., *Z. anorg. Chem.* **86**, 143 (1914); *C. A.* **8**, 1938 (1914); *J. Soc. Chem. Ind.* **33**, 352 (1914). Colorimetric estimation of small amounts of hydrogen sulfide. By action of  $\text{FeCl}_3$  and dimethyl-*p*-phenylenediamine sulfate upon an aqueous solution of  $\text{H}_2\text{S}$  containing free  $\text{HCl}$ , methylene blue is formed. Under given conditions the intensity of the color developed is proportional to the amount of  $\text{H}_2\text{S}$  present. Color stable for weeks. Define conditions necessary for the best results.
- ROSENKRÄNZER, F., *Z. anorg. Chem.* **87**, 319 (1914). On the speed of solution of zinc blend and galena in dilute sulfuric acid. Use the method of Mecklenburg and Rosenkränzer, *Z. anorg. Chem.* **86**, 143 (1914).
- SNELL, F. D., *Colorimetric Analysis*, p. 137, D. Van Nostrand Co., New York, 1921. Determination of hydrogen sulfide by methylene blue.
- HEATH, F. H. and LEE, F. A., *J. Am. Chem. Soc.* **45**, 1643 (1923); *J. Soc. Chem. Ind.* **42**, 853A (1923). Errors in the determination of hydrogen sulfide. Suggest the use of *p*-amino-dimethylaniline,  $\text{HCl}$  and  $\text{FeCl}_3$  in determining  $\text{H}_2\text{S}$  in mineral waters but report no results obtained by this method.
- ALMY, L. H., *J. Am. Chem. Soc.* **47**, 1381 (1925). A method for the estimation of hydrogen sulfide in proteinaceous food products. Uses *p*-amino-dimethylaniline hydrochloride and  $\text{FeCl}_3$ .  $\text{ZnAc}_2$  solution used to absorb the  $\text{H}_2\text{S}$ .

#### Hydrolysis.

- TIZARD, H. T., *Proc. Chem. Soc. (London)*, Oct. 20, 1910; *Chem. News* **102**, 277 (1910). The hydrolysis of aniline salts measured colorimetrically.
- HARDING, V. J. and MACLEAN, R. M., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.* **24**, xv (1916). A comparison of the Sørensen, Van Slyke, and colorimetric methods for the estimation of protein hydrolysis.

#### Hydroxylamine.

- SCHROEDER, H., *Chem. News* **109**, 205 (1914); *J. Chem. Soc.* **106**, ii, 486 (1914). The detection and estimation of hydroxylamine. Uses solid  $\text{Na}$  nitroprusside and  $\text{NaOH}$ . A characteristic magenta color develops.
- PUCHER, G. W. and DAY, H. A., *J. Am. Chem. Soc.* **48**, 672 (1926). A colorimetric method for the estimation of hydroxylamine. Method depends on the reaction of  $\text{NH}_4\text{OH}$  with  $\text{C}_6\text{H}_5\text{COCl}$  and  $\text{FeCl}_3$ .

#### Imidazole.

- KOESSLER, K. K. and HANKE, M. T., *J. Biol. Chem.* **39**, 497 (1919). A microchemical colorimetric method for estimating imidazole derivatives. Use *p*-phenyldiazonium sulfonate.
- HUNTER, G., *Biochem. J.* **19**, 42 (1925). Commercial Congo red and methyl orange must be purified before use in colorimetric work.
- LEITER, L., *J. Biol. Chem.* **64**, 125 (1925). Use method of Koessler-Hanke.

#### Indican.

- AUTENRIETH, W. and FUNK, A., *Münch. med. Wochschr.* **59**, 689; *C. A.* **6**, 2088 (1912). The colorimetric method for determining glucose and indican in urine, as also the iron in the blood.

ERUST, E., *Deut. Z. Chirurgie* **192**, 330 (1925); *Ber. ges. Physiol. exptl. Pharmacol.* **34** (1926). Indican determination in blood and kidney insufficiency. Details of a colorimetric indican determination.

#### Indigo.

KOPPESCHAAR, W. F., *Z. anal. Chem.* **38**, 1 (1899). Determination of indigo blue and indigo red in natural and synthetic indigo.

#### Indole.

HERTER, C. A. and FOSTER (Miss) M. L., *J. Biol. Chem.* **1**, 257 (1905-06). A method for the quantitative determination of indole. Use  $\beta$ -naphthaquinone-sodium-monosulfonate. Gives blue or green-blue color with faintly alkaline solution of indole. See *J. Exptl. Med.* **7**, No. 1, 1905, for a description of the reaction.

HERTER, C. A. and FOSTER, (MISS) M. L., *J. Biol. Chem.* **2**, 267 (1906). On the separation of indole from skatole and their quantitative determination. Use  $\beta$ -naphthaquinone-sodium-monosulfonate for indole and Ehrlich's aldehyde reagent for skatole after separation.

MARSHALL, W. E., *J. Hyg.* **7**, 581 (1907); *J. Chem. Soc.* **92**, ii, 995 (1907). The *p*-dimethylaminobenzaldehyde test for indole. Ehrlich's test. M. states method lends itself to colorimetric estimations.

MILLER, C. W. and TAYLOR, A. E., *J. Biol. Chem.* **17**, 531 (1914). Suggest that the reagent (30 g. ammonium molybdate and 25 cc.  $H_2SO_4$  dissolved in 1 liter of distilled water) might be developed to serve for the colorimetric estimation of the indole and phenol derivatives contained in urine.

NELSON, V. E., *J. Biol. Chem.* **24**, 527 (1916). Some color reactions for indole and skatole. Possibility of some quantitative methods.

NELSON, V. E., *J. Biol. Chem.* **24**, 533 (1916). Indole in cheese. Uses sodium-naphtha-quinone-monosulfonate.

BERGEIM, O., *J. Biol. Chem.* **32**, 17 (1917). The determination of fecal indole. Uses  $\beta$ -naphthaquinone-sodium-monosulfonate and KOH. Blue color formed.

ZOLLER, H. F., *J. Biol. Chem.* **41**, 25 (1920). Quantitative estimation of indole in biological media. Discusses several color reactions.

#### Inulin.

OKEY, R., *J. Biol. Chem.* **38**, 33 (1919). Studies on the behavior of inulin in the animal body. Application of the Benedict method to the estimation of levulose and inulin.

#### Iodine.

STRUVE, H., *Z. anal. Chem.* **8**, 230 (1869). Liberates iodine and dissolves it in  $CS_2$ .

BAUMANN, E. and ROOS, E., *Z. Physiol. Chem.* **21**, 489 (1895-96). Fuse substance with NaOH (using a little  $NaNO_3$  if much organic matter is present), dissolve melt in water, filter, acidify filtrate with  $H_2SO_4$ , add  $NaNO_3$ , shake out the liberated iodine with chloroform and compare the solution with standards.

- BOURCET, P., *Compt. rend.* **128**, 1120 (1899); *J. Chem. Soc.* **76**, 516 (1899); *J. Soc. Chem. Ind.* **18**, 613 (1899); *Z. anal. Chem.* **42**, 115 (1903). Detection and colorimetric estimation of traces of iodine in organic matter. Iodine liberated by nitrous acid in the presence of  $\text{CS}_2$ .
- SEIDELL, A., *J. Biol. Chem.* **3**, 391 (1907); *C. A.* **2**, 1251 (1908). Small amounts of iodine in organic matter determined by fusing latter with excess caustic alkali, dissolving and neutralizing, liberating iodine with  $\text{HNO}_2$  and absorbing the iodine in  $\text{CHCl}_3$ . Match against standard pink solutions of the dye Fuchsin S.
- RIGGS, L. W., *Proc. Am. Soc. Biol. Chem.*, *J. Biol. Chem.* **6**, xli (1909). The determination of iodine in protein combinations. Suggests improvements of Baumann's process.
- SEIDELL, A., *J. Biol. Chem.* **10**, 95 (1911-12). Comparative investigation of Hunter, Baumann, and Riggs modification of Baumann's method.
- CAMERON, A. T., *J. Biol. Chem.* **18**, 335 (1914). The distribution of iodine in plant and animal tissues. Considers accuracy and degree of sensibility of methods of Baumann (comparison with solution of known strength), Seidell (comparison with a standard solution of Fuchsin S), and Bourcet (addition of nitrite in presence of acid and  $\text{CS}_2$  and colorimetric comparison).
- KRAUSS, R. B., *J. Biol. Chem.* **22**, 151 (1915). The determination of iodine in the presence of organic matter. Based upon the fact that palladous chloride when added in the cold to a dilute solution of iodide gives a deep brown color due to the formation of palladous iodide. A difference of 0.01 mg. iodine in 100 cc. can easily be detected with the naked eye. Using a Dubosq colorimeter the range is increased 1 to 100.
- LEWIS, P. A. and KRAUSS, R. B., *J. Biol. Chem.* **22**, 159 (1915). Further observations on the presence of iodine in tuberculous tissues and in the thyroid gland. Use method given in *J. Biol. Chem.* **22**, 151 (1915).
- KRAUSS, R. B., *J. Biol. Chem.* **24**, 321 (1916). The electrolytic determination of iodine present in organic matter. Mentions colorimetric method [*J. Biol. Chem.* **22**, 151 (1915)] and claims it is much more sensitive than the electrolytic.
- BOHN, R. M., *J. Biol. Chem.* **28**, 375 (1916-17). The iodine content of food materials. Finds Krauss' method [*J. Biol. Chem.* **22**, 151 (1915)] unsatisfactory.
- SMITH, G. E., *J. Biol. Chem.* **29**, 215 (1917). Uses method of Hunter.
- DUBIEF, J., *Ann. fals.* **16**, 80 (1923); *J. Chem. Soc.* **124**, ii, 332 (1923). Rapid estimation of total iodine in mineral waters containing sulfides. Sulfides and organic matter oxidized by alkaline permanganate and halogens then liberated by acidifying with  $\text{H}_2\text{SO}_4$  and dissolved in  $\text{CS}_2$ . Excess permanganate removed with  $\text{H}_2\text{O}_2$ .
- ELLENBERG, T. v., *Biochem. Z.* **139**, 400 (1923).
- McCLENDON, J. F., *J. Biol. Chem.* **60**, 289 (1924). The determination of iodine in food, drink, and excreta. Extracts with  $\text{CCl}_4$ .
- LANGE, N. A. and WARD, L. A., *J. Am. Chem. Soc.* **47**, 1000 (1925); *C. A.* **19**, 1548. Determination of small amounts of iodine as iodide and iodate. Use  $\text{H}_2\text{O}_2$  and *o*-tolidine in alcohol. Bluish-green color obtained.

YOSHIMATSU, S., *Tôhoku J. Exptl. Med.* **8**, 107 (1926). Determination of iodine in urine. "AgI dissolved in KCN soln. is reduced by means of Na<sub>2</sub>S with the production of a dark brown colored soln. which is estd. colorimetrically. The method provides for the detn. of both Cl and I in the same sample of urine, and yields results which average within 3 per cent of the amts. actually present." L. W. Riggs, *C. A.* **21**, 1133 (1927).

#### Iron.

OSSIAN, H., *Pharm. Centralblatt* **1837**, 205. Early mention of KCNS as a qualitative test for Fe.

HARTING, P., *J. prakt. Chem.* **22**, 51 (1841); cf. Carnelley, *Chem. News* **30**, 259 (1874). States that 1 part of Fe (as sulfate) produces a color in 300,000 parts of water containing ferrocyanide.

HERAPATH, T. J., *J. Chem. Soc.* **5**, 27 (1852). Uses KCNS.

DAVIES, J., *Chem. News* **8**, 163 (1863); *Z. anal. Chem.* **3**, 370 (1864). Uses KCNS for Fe in waters.

HERAPATH, W., *Chem. News* **8**, 182 (1863). Note calling attention to the paper by T. J. Herapath, *Chem. Gazz.* **12**, 172, who used KCNS for estimating iron and gave a method of procedure "very superior" to the one recommended by J. Davies, *Chem. News* **8**, 163 (1863), who also uses KCNS.

RHEINECK, H., *Dingler's polytech. J.* **201**, 467 (1871). Uses ferrocyanide for Fe in liquids employed in dye works.

BÖTTGER, R., *Chem. Zentr.* **1874**, 407; *J. Chem. Soc.* **27**, 1101 (1874). Detection of iron in nickel salts. Uses KCNS.

MORRELL, T. T., *Am. Chemist* **4**, 287 (1874); *Z. anal. Chem.* **14**, 390 (1875). An approximate method based upon the liberation of I from KI by FeCl<sub>3</sub>. Resulting iodine solution matched against standard solutions.

MÜLLER, A., *Pogg. Ann. Ergänzungs-b.* **6**, 123 (1874). Found that an elevation of 30° increased the color intensity of a FeCl<sub>3</sub> solution from 1 to 1.4 or 1.5.

MÜLLER, A., *Pogg. Ann. Ergänzungs-b.* **6**, 262 (1874). Effect of HAc and HCl on FeCl<sub>3</sub> solutions. Both acids increase the intensity of FeCl<sub>3</sub> solutions. Increase due to HAc is only about 1/10 of that due to HCl.

CARNELLEY, T., *Chem. News* **30**, 257 (1874); *J. Chem. Soc.* **28**, 285 (1875). Uses K<sub>4</sub>Fe(CN)<sub>6</sub> for Fe in waters. Will detect 1 part of Fe in 13,000,000 parts of water.

VOGEL, A., *N. Rep. Pharm.* **25**, 180 and *Chem. Zentr.* **1876**, 375. On the delicacy of the salicylic acid test for Fe.

PAGLIANI, S., *Gazz. chim. ital.* **9**, 23; *J. Chem. Soc.* **36**, 748 (1879). Makes a quantitative study of the effects of H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, and HCl on the delicacy of the salicylic acid test for Fe.

SMITH, E. F., *Proc. Am. Phil. Soc.* **18**, 214 (1880). On the delicacy of the KCNS and salicylic acid tests for Fe. Salicylic acid is a "decidedly good reagent for iron in the presence of an excess of copper." It is more delicate than thiocyanate in such cases.

WAGNER, A., *Z. anal. Chem.* **20**, 349 (1881); *Chem. News* **45**, 35 (1882). On

- the delicacy of the ferrocyanide, thiocyanate, and tannic tests for Fe and the ferrocyanide, ammonia, and K xanthogenate tests for Cu.
- WERNER, H., *Z. anal. Chem.* **22**, 44 (1883); *J. Chem. Soc.* **44**, 510 (1883). Uses thiocyanate.
- SHILTON, A. J., *Chem. News* **49**, 234 (1884). Brief note on the reducing action of thiocyanate.
- THOMSON, A., *Chem. News* **51**, 259 (1885); *Proc. Chem. Soc.* May 21, 1885; *J. Chem. Soc.* **47**, 493 (1885). Uses thiocyanate. Ag and Cu and in some cases Co, are the only common metals that interfere. Clowes pointed out the necessity of having the amount of acid constant and that  $\text{HgCl}_2$  especially must be avoided.
- VENABLE, F. P., *J. Anal. Chem.* **1**, 312 (1887); *Chem. Zentr.* **1887**, i, 185. A qualitative test for Fe:  $\text{Co}(\text{NO}_3)_3$  in concentrated HCl gives a blue solution which turns green when Fe is added. 0.00003 g. of a ferric salt will produce a distinct green color.
- TATLOCK, R. R., *J. Soc. Chem. Ind.* **6**, 276, 352 (1887); in condensed form in *Analyst* **12**, 160 (1887). On the determination of minute proportions of iron, with special reference to alum and sulphate of alumina. Uses  $\text{NH}_4\text{CNS}$ . Extracts the ferric thiocyanate by ether and compares the color with ethereal layers of the same volume and thickness containing known amounts of Fe. Based on the observation of Natanson [*Ann.* **130**, 246 (1864)] that the reaction is more sensitive when ether is employed as solvent.
- COMBES, A., *Compt. rend.* **105**, 868 (1887). *J. Chem. Soc.* **54**, 128 (1888). Metallic derivatives of acetylacetone.
- SABANEJEFF and KISLAKOWSKY, *Chem. Zentr.* **1888**, 84; *Pharm. Ztg. Russ.* **26**, 776; *J. Chem. Soc.* **54**, 757 (1888). Use  $(\text{NH}_4)_2\text{S}$  for Fe in waters.
- BLOUNT, B., *Chem. News* **58**, 195 (1888); see also *J. Anal. Chem.* **3**, 75 (1889). Blount says use of Co indicator (*J. Anal. Chem.* **1**, 312; **2**, 4, 169) for Fe seems needless, since strong HCl gives yellow  $\text{FeCl}_3$ .
- BELL, J. C., *J. Soc. Chem. Ind.* **8**, 175 (1889); *J. Chem. Soc.* **58**, 419 (1890). Uses ferrocyanide for Fe in waters.
- JOLLES, A. F., *Chem.-Ztg. Rep.* **13**, 8; see also *J. Anal. Chem.* **3**, 84 (1889); *Z. anal. Chem.* **33**, Part 3. Uses  $\text{NH}_4\text{CNS}$  for Fe in mineral and river waters.
- KRÜSS, G. and MORAHT, H., *Ber.* **22**, 2061 (1889); *J. Chem. Soc.* **56**, ii, 1129 (1889). Double thiocyanates of iron and potassium.
- LAPICQUE, L., *Bull. soc. chim.* [3], **2**, 295 (1889); *J. Chem. Soc.* **58**, 297 (1890). Estimation of iron in blood. Uses  $\text{NH}_4\text{CNS}$ .
- LAPICQUE, L., *Compt. rend. soc. biol.* **1890**, 669; *J. Chem. Soc.* **62**, 240 (1892). Uses alkali thiocyanate for Fe in blood.
- RIBAU, J., *Bull. soc. chim.* [3], **6**, 996 (1891); *J. Chem. Soc.* **62**, 1132 (1892); *J. Soc. Chem. Ind.* **11**, 269 (1892). Presents numerous results on the determination of Fe in blood by the thiocyanate, acetate, and alkali tartrate methods which prove the methods inaccurate.
- MAGNANINI, G., *Z. phys. Chem.* **8**, 1 (1891). On the reaction between ferric salts and solutions of thiocyanates.

- VERNON, H. M., Chem. News **66**, 177, 191, 202, 214 (1892). On the reactions of ferric salts with thiocyanates.
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#### Isobutyl Alcohol.

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**Lactic Acid.**

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**Lactose.**

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**Lamp, Daylight.**

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**Lead.**

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- dilute to 20 cc., mix with 2 cc. of freshly prepared ammonium sulfide, and compare with that of standards of  $\text{PbSO}_4$  in aqueous  $\text{KOH}$  solution. Standard should contain 1 mg.  $\text{Pb}$  per cubic centimeter. Measured amounts are diluted to 20 cc.
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- EGELING, C. G., *Pharm. Weekblad* **44**, 338 (1907); *J. Chem. Soc.* **92**, ii, 398 (1907); *Apoth.-Ztg.* **22**, 275. Colorimetric estimation of lead in drinking water. Review of existing methods and description of a  $\text{K}_2\text{CrO}_4$  method.
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- KNAPP, A. W., *J. Soc. Chem. Ind.* **30**, 165 (1911); *Z. anal. Chem.* **51**, 587 (1912). The estimation of small quantities of lead in beer. Uses  $\text{HAc}$  solution of the sample and adds  $\text{H}_2\text{S}$ .
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- JÄRVINEN, K. K., *Z. Nahr. Genussm.* **45**, 183 (1923); *J. Chem. Soc.* **124**, ii, 655 (1923). Colorimetric estimation of small quantities of metals in food-stuffs and the preliminary destruction of the organic matter. Details for the destruction of the organic matter are given and for the estimation of Sn and Pb in the presence of one another, Cu and Zn in the presence of one another, Al, Ni, As, and Sb. Use  $\text{H}_2\text{S}$  or  $\text{Na}_2\text{S}$ .
- ANDREW, R. I., *Chem. News* **127**, 393 (1923). The colorimetric estimation of lead in cream of tartar. Uses KCN,  $\text{NH}_4\text{OH}$ , and  $\text{Na}_2\text{S}$ .
- THRESH, J. C., *Analyst* **49**, 124 (1924); *C. A.* **18**, 1627 (1924); *J. Soc. Chem. Ind.* **43**, B350 (1924). The estimation of lead in potable waters and in urine. Uses gelatine, acid solution and  $\text{H}_2\text{S}$ .
- ANDREW, R. I., *Analyst* **49**, 129 (1924); *C. A.* **18**, 1628 (1924). The colorimetric estimation of lead in cream of tartar. Uses KCN,  $\text{NH}_4\text{OH}$ , and  $\text{Na}_2\text{S}$ . Allen's Commercial Org. Anal., 4th ed., recommends clarifying a solution by passing through animal charcoal. This must *not* be done since 0.5 g. charcoal will adsorb 1 mg. Pb.
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- Standard Methods for the Examination of Water and Sewage*, 6 ed., p. 53, American Public Health Association, New York, 1925. Uses  $\text{HAc}$ ,  $\text{NH}_4\text{Ac}$ , and  $\text{H}_2\text{S}$ .



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- KLOSTERMANN, M., Naturwissenschaften **14**, 1116 (1926); C. A. **21**, 933 (1927). Determination of lead in organic materials. Colorimetric estimation of  $PbO_2$  was accomplished by means of tetramethyldiaminodiphenylmethane, which on oxidation gives a blue coloration. The sensitivity is about 0.005 mg.
- NECKE, A., SCHMIDT, P. and KLOSTERMANN, M., Deut. med. Wochschr. **52**, 1855 (1926). The determination of minute quantities of lead. "Minute quantities of Pb in the tissues of animals may be detn. colorimetrically. All org. matter is first destroyed with fuming  $HNO_3$  and  $H_2SO_4$ . The Pb, Fe, Mn and Cu are pptd. by  $H_2S$  and collected on a filter. After removal of the Fe and Mn by alc. and  $H_2SO_4$ , and of the Cu by KCN, the PbS is dissolved and oxidized by NaOCl. After filtration, tetramethyldiaminodiphenylmethane in AcOH soln. is added and the blue color formed is compared with a known standard." A. Grollman, C. A. **21**, 1284 (1927).
- BERNOUILLI, A. L., Helv. Chim. Acta. **9**, 827 (1926); C. A. **21**, 30 (1927). The sliding-gage colorimeter and the determination of minute quantities of ammonia, nitrite, lead, and iron. By means of this instrument it is possible to det. 0.002 mg. of Fe dissolved in 0.1 cc. with an accuracy of 0.4 of 1 per cent of the total amount present.
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#### Lecithin.

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- HERZFELD, E., Schweiz. med. Wochschr. **53**, 797 (1923); Chem. Zentr. **1924**, i, 2804; J. Chem. Soc. **126**, ii, 796 (1924). Method based on turbidity produced by adding a mixture of phosphotungstic acid and HCl to a dilute ethyl alcohol extract of the substance.
- GRIGAUT, A., Z. biol. Ges. **91**, 1014 (1924). Colorimetric method for lecithin determination in blood.
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#### Licopin.

- CONNELL, S. J. B., Biochem. J. **18**, 1127 (1924); J. Chem. Soc. **128**, i, 214 (1925). Uses a Stanford colorimeter and standard solutions of  $K_2Cr_2O_7$  and  $CoSO_4$  (Ni-free). Error less than 1 per cent.

Lipoids. (See **Lecithin**.)**Magnesium.**

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- KRAMER, B. and TISDALL, F. F., J. Biol. Chem. **47**, 475 (1921); Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **46**, xl (1921). Johns Hopkins Hospital Bull. **32**, 46 (1921). A simple technique for the determination of calcium and magnesium in small amounts of serum. Use  $\text{Fe}(\text{CNS})_3$ .
- KRAMER, B. and TISDALL, F. F., J. Biol. Chem. **48**, 228 (1921). Use  $\text{Fe}(\text{CNS})_3$ .
- SNELL, F. D., Colorimetric Analysis, p. 86, D. Van Nostrand Co., New York, 1921. Magnesium by determination of the phosphate as phosphomolybdate.
- BRIGGS, A. P., Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **50**, xlviii (1922). Uses phosphomolybdic acid.
- HAMMETT, F. S. and ADAMS, E. T., J. Biol. Chem. **52**, 211 (1922); C. A. **16**, 2342 (1922); J. Soc. Chem. Ind. **41**, 612A (1922). A modification of the Kramer and Tisdall method. Suitable for urine, blood, and tissue extracts. Mg separated as  $\text{MgNH}_4\text{PO}_4$ , dissolved in HCl and P estimated colorimetrically by the Bell and Doisy method.
- BRIGGS, A. P., J. Biol. Chem. **52**, 349 (1922); C. A. **16**, 2701 (1922). Uses hydroquinone upon  $\text{MgNH}_4\text{PO}_4$ .
- DENIS, W., J. Biol. Chem. **52**, 411 (1922). The determination of magnesium in blood, plasma, and serum.
- HAMMETT, F. S. and ADAMS, E. T., J. Biol. Chem. **54**, 565 (1922); C. A. **17**, 943 (1923); Analyst **48**, 92 (1923). Use the Bell-Doisy reaction.  $\text{MgNH}_4\text{PO}_4$  separated by centrifugation instead of filtration.
- GRÉGOIRE, A. and SOLA, T., Bull. soc. chim. Belg. **32**, 131 (1923); C. A. **17**, 1931 (1923); J. Soc. Chem. Ind. **42**, 427A (1923); Z. anal. Chem. **64**, 342 (1924). Mg oleate formed and color compared with a standard solution.
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- COLLIP, J. B. and CLARK, E. P., J. Biol. Chem. **64**, 485 (1925). Use Brigg's method.
- HAWK, P. B. and BERGEIM, O., Practical Physiological Chemistry, 9 ed. P. Blakiston's Son and Co., Philadelphia, 1926. Determination of magnesium in serum, pp. 410-411.

KRAMER, B. and HOWLAND, J., *J. Biol. Chem.* **68**, 715 (1926). Determination of Mg in bone.

### Manganese.

CRUM, W., *Ann.* **65**, 219 (1845). Suggests the use of  $\text{PbO}_2$  in  $\text{HNO}_3$  solution but gives no details.

HOPPE-SEYLER, J., *prakt. Chem.* **90**, 303 (1863). Proves spectroscopically that the color formed by  $\text{PbO}_2$  is due to permanganate.

PICHARD, P., *Compt. rend.* **75**, 1821 (1872); *Dingler's polytech. J.* **207**, 136 (1873); *Chem. News* **27**, 85 (1873); *Bull. soc. chim.* [2], **19**, 253; *Z. anal. Chem.* **12**, 308; *J. Chem. Soc.* **26**, 407; *Chem.-techn. Rep.* **12**, a, 195. Uses  $\text{PbO}_2$  in  $\text{HNO}_3$  solution, and gives details of method.

BRÜNNER, A., *Oesterr. Z. Berg.-Hüttenw.* **21**, 341 (1873); *Chem. Zentr.* **1873**, 757; *Bull. soc. chim.* [2], **21**, 278; *J. Chem. Soc.* **27**, 604, 816; *Chem.-techn. Rep.* **12**, b, 196; *Wagner's Jahresber.* **20**, 10; *Polytech. Centr.* **1873**, 1367; *Dingler's polytech. J.* **210**, 278. Schnell durch fñhrbare colorimetrische Probe auf Mangangehalt des Roheisens, Stahls, Eisens, und Erz. Converts Mn to sodium manganate by fusion with NaOH and compares solution with standards.

KOPPMAYER, M., *Dingler's polytech. J.* **211**, 133 (1874); *Chem. Zentr.* **1874**, 138; *J. Chem. Soc.* **27**, 1009; *Polytech. Centr.* **1874**, 395. Ueber A. Brñnner's colorimetrische Probe auf Mangangehalt des Stahls, Eisens, und Erz. Says the method is valueless.

MORRELL, T. T., *Am. Chemist* **5**, 213 (1874-75). Mn precipitated from an ammoniacal solution by bromine. KI and HCl then added.  $\text{MnO}_2$  dissolves yielding an iodine solution which has the same depth of color for the same amount of  $\text{MnO}_2$  and the same dilution. On a 1-g. sample, as little as 0.001 per cent of Mn tints the solution. Standard  $\text{MnCl}_2$  solutions give results differing by only 0.003 per cent.

MORRELL, T. T., *Am. Chemist* **6**, 45 (1875-76). Estimation of manganese in spiegeleisen. Method same as reported by M. in *Am. Chemist* **5**, 213 (1874-75). Error not over 0.25 per cent.

PETERS, S., *Chem. News* **33**, 35 (1876); *Dingler's polytech. J.* **221**, 486; *J. Chem. Soc.* **29**, 750; *Wagner's Jahresber.* **22**, 19; *Chem.-tech. Rep.* **15**, 480. On the estimation of manganese in iron and steel. Uses lead peroxide.

DESHAYES, V., *Bull. soc. chim.* [2], **36**, 121 (1881).

LEDEBUR, A., *Oesterr. Z. Berg.-Hüttenw.* **41**, 417 (1882); *Chem. Zentr.* **13**, 733 (1882); *Z. anal. Chem.* **22**, 607 (1883); *Ber.* **15**, 2926; *Wagner's Jahresber.* **29**, 15; *Stahl u. Eisen* **2**, 626; *Rep. anal. Chem.* **2**, 346; *J. Chem. Soc.* **44**, 242; *J. Soc. Chem. Ind.* **2**, 249; *Techn.-Chem. Jahrb.* **5**, 11; *Dingler's polytech. J.* **248**, 215; *Chem.-techn. Rep.* **21**, b, 211. Uses  $\text{PbO}_2$ .

GOETZ, *Dingler's Polytech. J.* **248**, 215 (1883). Uses lead peroxide.

OSMOND, M., *Bull. soc. chim.* [2], **43**, 66 (1885); *Chem. Zentr.* **1885**, 234; *J. Iron Steel Inst. London* **1885**, a, 275; *J. Chem. Soc.* **48**, 690; *Z. anal. Chem.* **25**, 552; *Chem. Ind.* **8**, 119; *Wagner's Jahresber.* **31**, 15; *Dingler's polytech. J.* **257**, 201; *Arch. Pharm.* **223**, 285; *Chem.-techn. Rep.* **24**, 249. Determination of

- Mn by means of  $\text{PbO}_2$  in the presence of metaphosphates and nitric acid. The  $\text{PbO}_2$  may be replaced by a current of ozonized oxygen.
- CHEEVER, B. W., *Trans. Am. Inst. Mining Eng.* **15**, 102 (1886); *J. Iron Steel Inst. London* **1885**, b, 736; *J. Anal. Chem.* **1**, 88. Colorimetric estimation of manganese in steel. Criticisms of the lead peroxide-nitric acid method.
- HUNT, A. E., *Trans. Am. Inst. Mining Eng.* **15**, 104 (1886); *J. Iron Steel Inst. London* **1886**, b, 1020; *J. Anal. Chem.* **1**, 89. The estimation of manganese in iron and steel. Uses lead peroxide and nitric acid.
- BABBITT, H. C., *Am. Chem. J.* **9**, 58 (1887); see also *J. Anal. Chem.* **1**, 331 (1887). Determination of manganese in steel and iron. Uses  $\text{Pb}_2\text{O}_4$ .
- MORGAN, J. J., *Chem. News* **56**, 82 (1887); *Chem. Zentr.* **1887**, 1268; *Z. Chem. Ind.* **1887**, b, 246; *Chem.-Ztg. Rep.* **11**, 219; *J. Chem. Soc.* **52**, 1140; *Wagner's Jahresber.* **33**, 271; *J. Anal. Chem.* **1**, 418; *Iron* **30**, 312; *Tech.-chem. Jahresber.* **10**, 16. Uses  $\text{PbO}_2$  in  $\text{HNO}_3$  solution for the determination of Mn in iron and steel.
- CHEEVER, B. W., *J. Anal. Chem.* **1**, 176 (1887). Conversion of manganese into permanganic acid. A study of the action of  $\text{PbO}_2$  in  $\text{HNO}_3$  solution.
- ROSSI, A. J., *Iron Age* **47**, 528; *J. Iron Steel Inst. London* **1891**, a, 443; **1892**, a, 491; *Stahl u. Eisen* **11**, 927; *Wagner's Jahresber.* **37**, 147. Uses sodium metaphosphate in the determination of Mn in iron, steel and cast iron.
- PARRY, J. and MORGAN, J. J., *Chem. News* **67**, 295 (1893); *Ind. and Iron* **1893**, 379; *Stahl u. Eisen* **13**, 898; *School Mines Quart.* **15**, 64.
- REDDROP, J. and RAMAGE, H., *J. Chem. Soc.* **67**, 275 (1895). The use of sodium bismuthate is suggested.
- FORESTIER, H., *Bull. soc. chim.* [3], **13**, 587 (1895). Uses lead peroxide or bismuth tetroxide.
- MIGNOT, A., *Rev. chim. anal. appl.* **4**, 329, 390 (1896); *Chem.-Ztg. Rep.* **20**, 234, 275. Uses lead peroxide or bismuth tetroxide in  $\text{HNO}_3$  solution.
- AUCHY, G., *J. Am. Chem. Soc.* **18**, 498 (1896); *Chem. Zentr.* **1896**, b, 208; *Chem. News* **74**, 214, 248, and 262; *J. Chem. Soc.* **70**, ii, 627; *School Mines Quart.* **18**, 43; *Eng. Mining J.* **61**, 111; *J. Soc. Chem. Ind.* **15**, 677; *Analyst* **21**, 335. Sources of error in volumetric and colorimetric determinations of Mn in steel.
- LEMAITRE, M., *Bull. soc. pharm. Bordeaux* **1897**, 268; *Chem. News* **76**, 219 (1897); *Ann. chim. anal. chim. appl.* **2**, 409. Determination of Mn in plants. Uses  $\text{PbO}_2$  and  $\text{HNO}_3$ .
- SCHNEIDER, L., *Chem.-Ztg.* **21**, 41 (1897); *Chem. Zentr.* **1897**, a, 436; *J. Iron Steel Inst. London* **1898**, a, 534; *J. Chem. Soc.* **74**, ii, 94; *Analyst* **22**, 110. Makes reference to colorimetric methods for determining Mn by oxidation to permanganic acid.
- PICHARD, P., *Compt. rend.* **126**, 550 (1898); *Chem. Zentr.* **1898**, a, 753; *Chem. News* **77**, 108; *Chem.-techn. Rep.* **37**, 286; *J. Soc. Chem. Ind.* **17**, 273; *Ann. chim. anal. chim. appl.* **3**, 123; *Z. anal. Chem.* **44**, 449 (1905). Uses lead peroxide and nitric acid.
- DUFFY, L., *Chem. News* **84**, 248 (1901). Gives full directions for the use of sodium bismuthate.

- MARSHALL, H., Chem. News **83**, 76 (1901); J. Chem. Soc. **80**, ii, 350 (1901). Uses K or  $\text{NH}_4$  persulfate.
- WALTERS, H. E., Chem. News **84**, 239 (1901); Proc. Eng. Soc. West. Penn. **17**, 257 (1901). Ammonium persulfate as a substitute for lead peroxide in the colorimetric estimation of manganese.
- WALTERS, H. E., Age of Steel, Nov. 1901, p. 23. Uses  $\text{PbO}_2$  and  $\text{NH}_4$  persulfate- $\text{AgNO}_3$  methods.
- TALBOT, H. P. and BROWN, J. W., A Bibliography of the Analytical Chemistry of Manganese 1785-1900, Smithsonian Miscellaneous Collections No. 1313, Washington, **1902**. The above references were checked against the colorimetric references given by T. and B. and several added which had been omitted. Also, a number of references to abstracts of papers listed above were added from the T. and B. Bibliography.
- MALETTE, J., La Révue technique **25**, 327 (1903); through Chem.-Ztg. Rep. **27**, 267 (1903); Analyst **28**, 371 (1903); J. Soc. Chem. Ind. **22**, 1209 (1903). Colorimetric estimation of manganese in steel. Uses  $\text{PbO}_2$  or  $\text{Pb}_2\text{O}_4$ .
- CLENNELL, J. E., Eng. Mining J. **78**, 827 (1904). Uses  $\text{HNO}_3$  and  $\text{PbO}_2$  in the determination of Mn in cyanide solutions.
- CRONER, F., Gesundh. Ing. **28**, 197 (1905); Chem. Zentr. **1905**, II, 74.
- TARUGI, N., Gazz. chim. ital. **36**, I, 332 (1906); J. Soc. Chem. Ind. **25**, 911 (1906). Oxidizes Mn to colored form in alkaline glycerine solution by air or hypochlorite. Will indicate as little as 0.005 gram of Mn.
- LÜHRIG, H. and BECKER, W., Pharm. Zentralhalle **48**, 137 (1907). Estimation of manganese in water. Tried several methods. Knorre's [Z. angew. Chem. **14**, 1149 (1901)] method found best. K. uses K persulfate used by Marshall [J. Chem. Soc. **59**, 771T (1891)].  $\text{MnO}_2$  estimated either gravimetrically or volumetrically. L. and B. found 0.0001 gram Mn per liter gave a pink coloration when a modification of Marshall's process was used.
- DUYK, M., Ann. chim. anal. chim. appl. **12**, 465 (1907); J. Soc. Chem. Ind. **27**, 248 (1908). Oxidizes Mn to  $\text{MnO}_4$  in slightly alkaline solution by hypochlorite, using  $\text{CuSO}_4$  as catalyzer.
- HOLLAND, P., Chem. News **96**, 3 (1907). Uses persulfate and silver nitrate.
- WESTON, R. S., J. Am. Chem. Soc. **29**, 1074 (1907); Chem. News **97**, 3 (1908). The determination of manganese in water. Uses Na bismuthate and filters the oxidized solution through a thoroughly washed asbestos filter in a Gooch crucible.
- KLUT, H., Mitt. kgl. Prüfungsanstalt Wasserversorgung und Abwässerbeseitigung **12**, 183; Z. anal. Chem. **50**, 726 (1911); C. A. **4**, 1071 (1910). Mentions almost all the methods for the determination of Mn in water. For quantitative work he recommends the  $\text{PbO}_2$  colorimetric method according to Volhard and Treadwell.
- SCHMIDT, M. R., J. Am. Chem. Soc. **32**, 965 (1910); C. A. **4**, 3054 (1910); J. Soc. Chem. Ind. **29**, 1085 (1910). Colorimetric determinations of manganese in the presence of iron. Method is applied to the determination of small amounts of Mn in pharmaceutical preparations. Makes use of Walter's method, Chem. News **84**, 239 (1901).

- DUMITRESCU and NICOLAU, *Ann. fals.* **3**, 370, 407 (1910). Use persulfate with a drop of  $\text{Co}(\text{NO}_3)_2$ .
- RODENBURG, J., *Chem. Weekblad* **7**, 877 (1910); *Chem. Zentr.* **1910**, II, 1504. Uses persulfate and silver nitrate.
- PRANDL, O. and CIVETTA, A., *Staz. sper. agrar. ital.* **44**, 58 (1911). Use  $\text{PbO}_2$ .
- GOTTFRIED, A., *Pharm. Zentralhalle* **1911**, 788; *Pharm. J.* **87**, 276 (1911); *J. Soc. Chem. Ind.* **30**, 1080 (1911). Determination of manganese in honey. Uses  $\text{NH}_4$  persulfate and  $\text{AgNO}_3$ .
- BERTRAND, G., *Bull. soc. chim. [4]* **9**, 361 (1911); *J. Soc. Chem. Ind.* **30**, 650 (1911). Uses K persulfate and silver nitrate.
- GORTNER, R. A. and ROST, C. O., *J. Ind. Eng. Chem.* **4**, 522 (1912); *Z. anal. Chem.* **52**, 586 (1913). Use sodium bismuthate and indicate objections to persulfate.
- The Chemist's Committee of the U. S. Steel Corporation, *J. Ind. Eng. Chem.* **4**, 807 (1912). Uses ammonium persulfate and silver nitrate.
- HAAS, F., *Z. Nahr. Genussm.* **25**, 392 (1912); *J. Soc. Chem. Ind.* **32**, 447 (1913). Uses persulfate and silver nitrate.
- STANICHTCH, *Rev. métal.* **8**, 891; *C. A.* **6**, 1264 (1912); *J. Soc. Chem. Ind.* **31**, 75 (1912). Rapid colorimetric determination of manganese in iron and steel by means of ammonium persulfate.
- DITTRICH, M., *Z. anorg. Chem.* **80**, 171 (1913); *J. Chem. Soc.* **104**, ii, 344 (1913); *J. Soc. Chem. Ind.* **32**, 383 (1913). The estimation of small quantities of manganese and chromium in minerals and rocks. Uses  $\text{NH}_4$  persulfate and  $\text{AgNO}_3$  for Mn and chromate method for Cr, after removal of Ag with NaCl.
- SCHOWALTER, E., *Z. Nahr. Genussm.* **26**, 104 (1913). Uses persulfate and silver nitrate.
- HARTWIG, L. and SCHELLBACH, H., *Z. Nahr. Genussm.* **26**, 439 (1913); *J. Soc. Chem. Ind.* **32**, 1125 (1913). Use ammonium persulfate and silver nitrate.
- JARDIN, F. and ASTRUC, A., *Compt. rend.* **157**, 338 (1913); *J. Soc. Chem. Ind.* **32**, 880 (1913). Manganese in drinking and mineral waters.
- LÜHRIG, H., *Chem.-Ztg.* **38**, 781 (1913); *J. Soc. Chem. Ind.* **33**, 709 (1914). Uses ammonium persulfate and silver nitrate.
- TILLMANS, J. and MILDNER, H., *J. Gasbel.* **57**, 496, 523, 544 (1914); *C. A.* **8**, 3085 (1914). Use persulfate and silver. Suggest adding  $\text{KIO}_4$ , acetic acid and "tetramethyl base" as a qualitative test. Blue color indicates Mn.
- SCHOWALTER, E., *Z. Nahr. Genussm.* **27**, 553 (1914); *J. Chem. Soc.* **108**, ii, 492 (1914). Uses  $\text{NH}_4$  persulfate and  $\text{AgNO}_3$ .
- HORVÁTH, BÉLA VON, *Z. anal. Chem.* **53**, 581 (1914). Uses ammonium persulfate and silver nitrate.
- SACHER, J. F., *Chem.-Ztg.* **39**, 319 (1915); *C. A.* **9**, 2043 (1915). Adds NaOH, lets  $\text{Mn}(\text{OH})_2$  oxidize in air and adds oxalic acid. Red color thus formed is due to a double salt.
- BARDACH, F., *Chem.-Ztg.* **39**, 457 (1915).
- SACHER, J. F., *Chem.-Ztg.* **39**, 458 (1915).
- DOBBIN, L., *Chem. News* **113**, 133 (1916). Historical review of persulfate and  $\text{PbO}_2$  methods.

- SACHER, J. F., *Chem. Zentr.* **1916**, I, 438; *Farben-Ztg.* **20**, 1309 (1915); *J. Chem. Soc.* **110**, ii, 451 (1916). Application of NaOH,  $\text{Mn}(\text{OH})_2$ , and  $\text{H}_2\text{C}_2\text{O}_4$  method to paints, pigments, varnishes, etc.
- WILLARD, H. H. and GREATHOUSE, L. H., *J. Am. Chem. Soc.* **39**, 2366 (1917); *C. A.* **11**, 3189 (1917); *J. Soc. Chem. Ind.* **37**, 41A (1918). Colorimetric determination of manganese by oxidation with periodate. There is appended a fairly complete bibliography (to 1916) of the colorimetric methods for the determination of Mn. The references are listed chronologically and with brief remarks. All are included in the present bibliography.
- TREADWELL, F. P. and HALL, W. T. (Translator from the German), *Analytical Chemistry*. Vol. II. Quantitative Analysis, 5 ed., p. 127, John Wiley & Sons, Inc., New York, **1919**. Uses  $\text{PbO}_2$  and persulfate- $\text{AgNO}_3$  methods.
- WESTER, D. H., *Rec. trav. chim.* **39**, 414 (1920); *J. Chem. Soc.* **118**, ii, 451 (1920). Determination of Manganese in plant ash. Marshall's persulfate method only one of four examined found satisfactory.
- SNELL, F. D., *Colorimetric Analysis*, p. 78, D. Van Nostrand Co., New York, **1921**. Determination of manganese as permanganate, oxidation by persulfate.
- SNELL, F. D., *ibid.*, p. 80, **1921**. Determination of manganese, oxidation by periodate.
- HESLINGA, J., *Chem. Weekblad* **19**, 302 (1922); *J. Chem. Soc.* **122**, ii, 660 (1922); *C. A.* **16**, 3283 (1922); *J. Soc. Chem. Ind.* **41**, 635A (1922). Colorimetric estimation of manganese in steels, alloys, and ores. Trivalent elements removed and Mn oxidized to hydrated  $\text{MnO}_2$  by means of  $\text{H}_2\text{O}_2$  and KOH. Yellowish-brown to dark brown colloidal solution of hydrated  $\text{MnO}_2$  is thus obtained.
- DENIGÈS, G., *Compt. rend.* **175**, 1206 (1922). The approximate estimation of magnesium in a single drop of sea-water. Uses K hypiodite.
- FORESTIER, H., *Bull. soc. chim.* **33**, 659 (1923); *C. A.* **17**, 2688 (1923); *J. Soc. Chem. Ind.* **42**, 780A (1923). Improvement in the colorimetric determination of manganese in steel. Uses the persulfate method.
- COLLINS, W. D., and FOSTER (Miss) M. D., *Ind. Eng. Chem.* **16**, 586 (1924); *C. A.* **18**, 2052 (1924). The determination of manganese in water by the sodium bismuthate method.
- SCOTT, W. W., *Standard Methods of Chemical Analysis*, 4 ed., p. 305, D. Van Nostrand Co., New York, **1925**. Uses  $(\text{NH}_4)_2\text{S}_2\text{O}_8$  and  $\text{AgNO}_3$ .
- Standard Methods for the Examination of Water and Sewage*, 6 ed., pp. 50-52, American Public Health Association, New York, **1925**. Uses the persulfate and the bismuthate methods.
- MCCRACKAN, R. F. and PASSAMANECK, E., *Arch. Path. Lab. Med.* **1**, 585 (1926); *C. A.* **20**, 2173 (1926). Manganese in urine. Its detection and determination.

### Mercury.

- VIGNON, L., *Compt. rend.* **116**, 584 (1893); *J. Soc. Chem. Ind.* **12**, 627 (1893). The estimation of mercury in dilute solutions.

- KONINCK, L. L. DE, *Rev. univ. des Mines et de la Metallurgie* [3], **23**, No. 3; *Chem. News* **69**, 13 (1894). Determination of mercury in dilute solutions of sublimate. Uses  $\text{H}_2\text{S}$ .
- BUDDEN, E. R. and HARDY, H., *Analyst* **21**, 12 (1896). Find the colorimetric methods preferable to electrolytic methods for testing beverages for metals when several are present. Metals tested: Cu, Pb, Hg.
- ESCHBAUM, F., *Chem. Zentr.* **1902**, I, 1133; *J. Chem. Soc.* **82**, ii, 476 (1902); *Pharm. Ztg.* **47**, 260 (1902). Colorimetric estimation of mercury in urine. Based upon turbidity of  $\text{HgCl}$  solution.
- SCHUMACHER, II, and JUNG, W., *Z. anal. Chem.* **41**, 471, 482 (1902); *Analyst* **27**, 368 (1902). A new colorimetric method of determining mercury in urine. Use  $\text{H}_2\text{S}$  solution.
- CARRÁCIDO, J. R., *Anal. soc. españ. fis. quim.* **4**, 314 (1906); *J. Chem. Soc.* **92**, ii, 131 (1907). Rapid estimation of mercuric chloride in very dilute solutions. Uses ammonia. Will detect as little as 1 part  $\text{HgCl}_2$  in 40,000 parts of solution.
- HEINZELMANN, A., *Chem.-Ztg.* **35**, 721 (1911); *C. A.* **5**, 3211 (1911). The colorimetric determination of mercury in urine. H. has made a critical study of the method of Schumacher and Jung (*Z. anal. Chem.* **41**, 482) and recommends how it should be carried out. Uses  $\text{H}_2\text{S}$ . Compares color of the colloidal  $\text{HgS}$  solution.
- PROCTER, H. R., and SEYMOUR-JONES, R. A., *J. Soc. Chem. Ind.* **30**, 404 (1911); *C. A.* **5**, 2231 (1911). The estimation of soluble mercuric salts at great dilutions. Based upon the formation of a colloidal solution of  $\text{HgS}$  by adding  $\text{H}_2\text{S}$  to the  $\text{Hg}$  solution containing 1 per cent  $\text{HAc}$ . Differences of 1 in 100,000 can be detected. A Schmidt and Haensch dipping colorimeter is used.
- AUTENRIETH, W. and MONTIGNY, W., *Münch. med. Wochschr.* **67**, 928 (1920); from *Chem. Zentr.* **1920**, iv, 426; *J. Chem. Soc.* **118**, ii, 773 (1920); *C. A.* **15**, 541 (1920). Estimation of mercury in urine. Estimated as  $\text{HgS}$ .
- BOOTH, H. S. and SCHREIBER, N. E., *J. Am. Chem. Soc.* **47**, 2625 (1925). Determine the sensitivity of the following colorimetric tests for  $\text{Hg}$ : (1)  $\text{H}_2\text{S}$ , (2)  $\text{SnCl}_2$ , (3)  $\text{KI}$ , (4)  $\text{NH}_4\text{CNS}$ , (5)  $\text{NH}_4$  thio-acetate, (6) acetylene, (7) phenylthiohydantonic acid, (8) diphenylcarbazine, (9) aniline, (10) tannic acid, (11) Mayer's reagent for alkaloids. The most sensitive reagents were (1), (2), (4), (6), (7), and (8). These gave positive tests at 5 parts per million. A new electromicroqualitative test which is easily sensitive to 1 part  $\text{Hg}$  ion per billion is described.
- STOCK, A. and POHLAND, E., *Z. angew. Chem.* **39**, 791 (1926); *C. A.* **20**, 3144 (1926). The colorimetric determination of very small quantities of mercury. The  $\text{Hg}$ -salt solution, contained in a small test tube, is treated with 4 drops of a saturated ethyl alcohol solution of diphenylcarbazine,  $\text{OC}(\text{NH}\cdot\text{NH}\cdot\text{C}_6\text{H}_5)_2$ , and direct comparison made with similar solutions containing known amounts of  $\text{HgCl}_2$ . 0.0005 mg. of  $\text{Hg}$  in 2 cc. of solution gives a distinct blue-violet coloration. If 0.01 mg. of  $\text{Hg}$  is present, the solution becomes almost opaque. The color disappears within a few hours in sunlight, with the formation of a precipitate, but remains for several days



if kept in the dark. The following substances interfere with the test: Zn, Fe, Co, Ni, Pb, Cu, Ag, Au, cyanides, bromides, and iodides.

### Methemoglobin.

STADIE, W. C., *J. Biol. Chem.* **41**, 237 (1920). A method for the determination of methemoglobin in blood. Uses KCN.

MCCELLROY, W. S., *J. Biol. Chem.* **42**, 297 (1920). A method for the determination of methemoglobin and hemoglobin in blood. Uses  $K_3Fe(CN)_6$ .

### Molybdenum.

BRAUN, A. D., *Z. anal. Chem.* **6**, 86 (1867). Originated the use of the blood-red color of molybdenum thiocyanate as a delicate test for Mo. Used Zn in HCl solution to reduce the Mo and concentrated the Mo thiocyanate by shaking out with ether.

SCHÖNN, Z. anal. Chem. **9**, 41, 330 (1870). First to observe the yellow color produced by the action of  $H_2O_2$  on molybdic and titanous acids.

BETTEL, W., *Chem. News* **97**, 40 (1908); *C. A.* **2**, 1248 (1908). The solution is evaporated almost to dryness, neutralized with  $HNO_3$  or  $H_2SO_4$  if alkaline and  $H_2O_2$  added. If yellow color appears, add a small drop of dilute ammonia. If Mo is present, a brownish-red color appears. 0.001 mg.  $MoO_3$  can be detected in a few cubic centimeters.

SURGE, G., *Chem. Eng. Mining Rev.* **11**, 258 (1919); *C. A.* **13**, 2322 (1919). Colorimetric estimation of molybdenite in low-grade ores and tailings. Uses tannic acid.

KLEINMANN, H., *Biochem. Z.* **99**, 42 (1919). Studies the influence of  $NH_4Cl$ , NaCl, HCl, and  $H_2SO_4$  on the colorimetry of P, Mo, and V compounds.

MALOWAN, S. L., *Z. anorg. Chem.* **198**, 73 (1919). Determination of molybdenum in iron and steel. Uses absolute alcoholic KOH solution and saturates with  $CS_2$  (this forms xanthic acid). Red coloration produced with Mo. Methods of the Chemists of the United States Steel Corporation for the Sampling and Analysis of Alloy Steels **1921**, 2d ed., p. 72.

KING, W. J., *Ind. Eng. Chem.* **15**, 350 (1923); *J. Soc. Chem. Ind.* **42**, 459A (1923). Estimation of small quantities of molybdenum in tungsten. Method based on the formation of a blood-red coloration of molybdenum thiocyanate. Method used when amount of Mo is less than 300 parts per million. Modifies and refines the method of L. Leley of the Philips' Lamp Works, Holland.

MEULEN, H. TER, *Chem. Weekblad* **22**, 80 (1925); *J. Chem. Soc.* **128**, ii, 330 (1925); *C. A.* **19**, 1390; *J. Soc. Chem. Ind.* **44**, B270 (1925). Method based on the dark red color of  $(NH_4)_2MoS_4$ . Method is rapid and fairly accurate, and not affected by alkalis. V and W interfere.

MAAG, O. L. and MCCOLLAM, C. H., *Ind. Eng. Chem.* **17**, 524 (1925). Rapid determination of molybdenum in steel. Use KCNS and  $SnCl_2$ .

WENDEHORST, E., *Z. anorg. allgem. Chem.* **144**, 319 (1925); *J. Soc. Chem. Ind.* **44**, B573 (1925). "Colloidal  $MoS_2$  has a brownish red to light yellow color which can be used as the basis for the colorimetric detn. of Mo. By means of the method a sample of com.  $MoO_3$  assayed the same as by the usual

gravimetric method. For the colorimetric standard,  $\text{MoO}_3$  can be prepd. by careful roasting of the freshly pptd. sulfide. Dissolve 0.05–0.1 g. of the oxide in water and a little  $\text{NH}_4\text{OH}$ . Boil off the excess of the latter and dil. to 500 cc. in a measuring flask. Take 15–20 cc. dil. with an equal vol. of water and the same amt. or a little more of  $\text{H}_2\text{S}$  water contg. 5 per cent of glycerol. Finally mix with an equal vol. of 0.2 N  $\text{H}_2\text{SO}_4$ , adding the acid until the red color no longer deepens. In the analysis, treat the ppt. of  $\text{MoS}_3$ , obtained in the usual way, with Br water to oxidize all the S and remove the excess  $\text{Br}_2$  by boiling. Then treat it in the same way as the standard. Solns. contg. only 0.01 g. Mo per l. can be analyzed in this way." W. T. H. C. A. **19**, 2614 (1925).

FUNCK, A. D., Z. anal. Chem. **68**, 283 (1926); C. A. **21**, 36 (1927). The method is based on the formation of reddish brown salts of permolybdic acid by the action of  $\text{H}_2\text{O}_2$  on molybdates in alkaline solution.

### Morphine.

IIINSDALE, S. J., Chem. News **62**, 77 (1890); see also J. Anal. and Appl. Chem. **5**, 107 (1891). Colorimetric method for estimating the morphine strength of laudanum and other preparations of opium. Uses  $\text{K}_3\text{Fe}(\text{CN})_6$  solution containing a little  $\text{FeCl}_3$ . Said to detect 0.001 mg. morphine. One minute is required to form a blue color.

PALMER, J. D., Merck's Report, **11**, 191 (1902).

RADULESCU, D., Bull. soc. sci. Bucuresti **14**, 602 (1905); through Pharm. J. **76**, 501 (1906); Analyst **31**, 234 (1906). Uses  $\text{NaNO}_2$  and sufficient acid to liberate the  $\text{HNO}_2$ . Before effervescence stops, add excess  $\text{KOH}$ . Morphine gives a pale rose to ruby-red tint, according to amount of morphine present. Reaction stated to be "peculiar" to morphine.

GEORGES, L. and GASCARD, J. pharm. chim. **23**, 513 (1906); J. Chem. Soc. **90**, ii, 507 (1906); J. Soc. Chem. Ind. **25**, 779 (1906). Use iodic acid.

MAI, C. and RATH, C., Arch. Pharm. **244**, 300 (1906); J. Chem. Soc. **90**, ii, 817 (1906); J. Soc. Chem. Ind. **27**, 828 (1908). Use Marquis' reagent (2 drops 40 per cent formaldehyde solution mixed with 3 cc.  $\text{H}_2\text{SO}_4$ ). As little as 0.00003 gram of morphine can be estimated.

SANGER, C. R. and BOUGHTON, W. A., Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **7**, xxxvii (1909–10). The estimation of morphine in cases of poisoning. Uses formaldehyde and concentrated  $\text{H}_2\text{SO}_4$ .

DENIGÈS, G., Bull. soc. pharm. Bordeaux **50**, 465; C. A. **5**, 567 (1911). Adds  $\text{H}_2\text{O}_2$  to morphine-HCl followed by  $\text{NH}_4\text{OH}$  and  $\text{CuSO}_4$  solutions, agitating after each addition. A rose to deep red color appears soon after the Cu salt is added. The depth of the color depends upon the concentration of the alkaloid. Roughly quantitative by suitable controls. Sensitive to a few hundredths milligram of alkaloid.

FABINYI, R., Oesterr. Chem. Ztg. **15**, 61; C. A. **6**, 1340 (1912); J. Soc. Chem. Ind. **31**, 298 (1912). Colorimetric determination of morphine and colchicine. Uses Radulescu's reaction which he makes quantitative for morphine. Reaction consists in acidifying solution with dilute  $\text{H}_2\text{SO}_4$ , adding a little  $\text{NaNO}_2$ ,

- (crystals) and after gas stops coming off, make alkaline. Pink to ruby-red color. Colchicine determination based on the dark olive-green color resulting upon boiling the solution and adding  $\text{FeCl}_3$ .
- FABINYI, R., Verh. Ges. deut. Naturforsch. Aerzte, **1912**, ii, 228; J. Chem. Soc. **102**, ii, 504 (1912). Makes Radulescu's reaction quantitative. Uses solid  $\text{NaNO}_2$  and concentrated  $\text{KOH}$ . Red color produced.
- HEIDUSCHKA, A. and FAUL, M., Arch. Pharm. **255**, 172 (1917); J. Chem. Soc. **112**, ii, 554 (1917); J. Soc. Chem. Ind. **36**, 1287 (1917). Colorimetric methods for the estimation of very small quantities of morphine. (1) Employ a modification of the Georges and Gascard method. One part in 12,500 can be detected, but quantitative measurements can only be made between 1 in 1500, and 1 in 5500. Method made sensitive to 1 in 18,500 by adding a little ammonia and estimations can be made between 1 in 5000 and 1 in 16,500. (2) Employ Marquis' reagent (2-3 drops of 40 per cent formaldehyde and 3 cc. concentrated  $\text{H}_2\text{SO}_4$ ). Morphine can be estimated between 1 in 1400 and 1 in 14,000 and detected at 1 in 25,000.
- MORGULIS, S. and LEVINE, V. E., J. Lab. Clin. Med. **5**, 321 (1919-20). A simplified method for the detection and estimation of the distribution of morphine. Use Lafon's reagent (a solution of a selenite or selenic acid, 0.5 per cent in concentrated  $\text{H}_2\text{SO}_4$ ) which gives a blue and finally fairly persistent olive-green color with morphine. Gives only relative quantitative results.
- GAUSS, H., J. Lab. Clin. Med. **8**, 699 (1920-21); C. A. **16**, 427 (1922). A colorimetric method for the estimation of morphine in colloidal mixtures and tissues. Uses Marquis' reagent (a mixture of 1 part 40 per cent formaldehyde and 15 parts of concentrated  $\text{H}_2\text{SO}_4$ ) which gives a purple-red color with morphine. This color gradually but slowly changes to violet and then to a fairly permanent blue.
- NICHOLLS, J. R., Analyst **47**, 509 (1922). Uses  $\text{H}_2\text{SO}_4$ - $\text{KIO}_3$  method of Georges, Analyst **31**, 265 (1906).

### Nessler's Reagent.

- SCHNEIDER, A., Pharm. Zentr. **50**, 546; C. A. **3**, 2421 (1909). Preparation of Nessler's reagent.
- FRERICHS, G. and MANNHEIM, E., Apoth. Ztg. **29**, 972 (1914). A recent and highly approved method of preparing Nessler's reagent.
- FRIEDRICHS, O. v., Arch. Pharm. **259**, 158 (1921); C. A. **16**, 786 (1922). Conditions for the suitable preparation of Nessler's reagent for pharmacopeias. Of 10 methods of preparation considered, the method of F. is believed to be the best, viz.: Shake 2 g.  $\text{KI}$  and 3.5 g. finely powdered  $\text{HgI}_2$  with 3 cc. water, add 60 cc. 0.2 N  $\text{KOH}$ , then dilute to 100 cc. with water. After several days decant off the clear liquid, or pass it through asbestos.
- KOCH, F. C. and McMEEKIN, T. L., J. Am. Chem. Soc. **46**, 2066 (1924). A new direct Nesslerization microkjeldahl method and a modification of the Nessler-Folin reagent for ammonia.
- RICHMOND, H. D., Analyst **60**, 67 (1925); C. A. **19**, 2610 (1925). Preparation of Nessler's solution.

RICHMOND, H. D., *Analyst* **50**, 336 (1925); *C. A.* **19**, 2921 (1925). Preparation of Nessler's solution.

WINKLER, L. W., *Z. Nahr. Genussm.* **49**, 163 (1925); *C. A.* **19**, 2314 (1925). Nessler's reagent without potassium iodide.

### Nickel.

WAGNER, R., *J. prakt. Chem.* **61**, 129 (1854); *Z. anal. Chem.* **5**, 425 (1866).

WINKLER, C., *J. prakt. Chem.* **97**, 414 (1866); *Z. anal. Chem.* **5**, 425 (1866).

SPÜLLER, J., *Chem.-Ztg.* **21**, 243 (1897); *J. Soc. Chem. Ind.* **16**, 470 (1897). Estimation of nickel in nickel steels. Dissolves sample in  $\text{HNO}_3$ , boils off fumes, precipitates Fe with  $\text{ZnO}$ , filters and matches the green colored filtrate against a standard Ni solution.

LUCAS, M., *Bull. soc. chim.* **21**, 432 (1899); *J. Chem. Soc.* **76**, ii, 614 (1899). *J. Soc. Chem. Ind.* **18**, 709 (1899). Uses K or  $\text{NH}_4$  thiocarbonate.

PIEBER, R., *Chem.-Ztg.* **24**, 393 (1900); *J. Chem. Soc.* **78**, ii, 628 (1900); *J. Soc. Chem. Ind.* **19**, 563 (1900). Colorimetric estimation of nickel in steel. Ammoniacal Ni-steel solutions used as standards. Results approximate.

CHALLINOR, R. W., *J. Roy. Soc., New South Wales*, **38**, 406 (1905); *J. Chem. Soc.* **94**, ii, 988 (1908).

TSCHUGAEFF, L., *Chem.-Ztg. Rep.* **29**, 247 (1905); *Analyst* **30**, 352 (1905). A new sensitive reagent for nickel. Uses  $\alpha$ -dimethylglyoxime. Excess free acid first removed by addition of  $\text{NH}_4\text{OH}$  or  $\text{NaAc}$ .

TSCHUGAEFF, L., *Ber.* **38**, 2520 (1905); *Analyst* **30**, 378 (1905). Uses  $\alpha$ -dimethylglyoxime.  $\text{NH}_4\text{OH}$  or  $\text{NaAc}$  first added to test solution to remove excess of free acid.

ARMIT, H. W. and HARDEN, A., *Proc. Roy. Soc.* **77**, B, 420 (1906); *J. Chem. Soc.* **90**, ii, 397 (1906); *J. Soc. Chem. Ind.* **25**, 498 (1906); cf. L. Tschugaeff, *Ber.* **38**, 2520 (1905). Estimation of small quantities of nickel in organic substances. Use  $\alpha$ -dimethylglyoxime method. Will detect 1 part Ni in 2,000,000 parts of water [Tschugaeff, *Compt. rend.* **145**, 679 (1907)].

MILBAUER, J., *Z. anal. Chem.* **46**, 656 (1907); *C. A.* **2**, 972 (1908). Colorimetric comparison of solutions of copper and nickel salts. Uses  $\text{NH}_4\text{OH}$ .

LEHMANN, K. B., *Arch. Hyg.* **68**, 423 (1911); *Z. anal. Chem.* **50**, 313 (1911). Determination of nickel in food and animal organs. Uses  $\text{K}_2\text{CS}_3$ .

ATAK, F. W., *Chem.-Ztg.* **37**, 773 and *Analyst* **38**, 316 (1913); *Z. anal. Chem.* **53**, 620 (1914). On the use of  $\alpha$ -benzildioxime for testing and determining small amounts of nickel.

HÜTTNER, C., *Z. anorg. Chem.* **86**, 341 (1914); *J. Soc. Chem. Ind.* **33**, 614 (1914); *Z. anal. Chem.* **54**, 471 (1915). Matches  $\text{HCl}$  solutions of the chlorides. *C. A.* **8**, 2540 (1914), gives a long abstract of the paper.

LINDT, V., *Z. anal. Chem.* **53**, 165 (1914); *J. Chem. Soc.* **106**, ii, 298 (1914); *J. Soc. Chem. Ind.* **33**, 335 (1914). Adds K thiocarbonate to the ammoniacal solution of Ni.

SNELL, F. D., *Colorimetric Analysis*, p. 73, D. Van Nostrand Co., New York, **1921**. Determination of nickel by potassium thiocarbonate.

- SNELL, F. D., *ibid.*, p. 74, 1921. Determination of nickel as the chloride in concentrated HCl.
- HACKL, O., Chem.-Ztg. **46**, 385 (1922); C. A. **16**, 2280 (1922). Detection and determination of small quantities of nickel and cobalt in silicate rocks. Ni by dimethylglyoxime. Co by nitroso- $\beta$ -naphthol or Vogel's test with  $\text{SCN}^-$ .
- JÄRVINEN, K. K., Z. Nahr. Genussm. **45**, 183 (1923); J. Chem. Soc. **124**, ii, 655 (1923). Colorimetric estimation of small quantities of metals in foodstuffs and the preliminary destruction of the organic matter. Details for the destruction of the organic matter are given and for the estimation of Sn and Pb in the presence of one another, Cu and Zn in the presence of one another, Al, Ni, As, and Sb. Use  $\text{H}_2\text{S}$  or  $\text{N}_4\text{S}$ .
- ROLLET, A.-P., Compt. rend. **183**, 212 (1926); C. A. **20**, 3274 (1926). Uses Br,  $\text{NH}_4\text{OH}$ , and dimethylglyoxime solution in alcohol. Quantities of Ni varying from 0.001 to 0.01 mg. can be determined with an accuracy of about 5 per cent of the Ni content. Details are given for applying the method to Co salts, steel and organic material.
- FAIRHALL, L. T., J. Ind. Hyg. **8**, 528 (1926); C. A. **21**, 874 (1927). The colorimetric determination of minute amounts of nickel. Potassium dithio-oxalate as a sensitive reagent. In biologic studies concerning Ni ingestion, it was found possible to determine 1 mg. of Ni with an accuracy of 1 per cent by a colorimetric method based on the formation of magenta-colored Ni dithio-oxalate. The presence of alkali and alkaline earth cations does no harm and the color develops in neutral or in acid solutions.

#### Niobium. (See Columbium.)

#### Nitrate (and Nitric Acid).

- KERSTING, R., Ann. Chem. Pharm. **125**, 254 (1863); Chem. News **8**, 186 (1863). Detection of nitric acid in potable water by means of brucine.
- SPRENGEL, H., Pogg. Ann. **121**, 188 (1864). First to propose the use of phenol sulfonic acid for the detection and determination of nitrates.
- KEKULÉ, Jahresber. **1866**, p. 447. Uses phenolsulfonic acid.
- KEKULÉ and LEVERKUS, Z. Chem. **1866**, p. 693. Use phenolsulfonic acid.
- KEKULÉ, Z. Chem. **10**, 199, 641 (1867); Lehrbuch, III, 236; Jahresber. **1867**, p. 637. Uses phenolsulfonic acid.
- NESSLER, J., Z. anal. Chem. **7**, 415 (1868).
- CHAPMAN, E. T., J. Chem. Soc. **21**, 172 (1868).
- KEKULÉ, A., Ber. **2**, 330 (1869); Z. Chem. **1869**, p. 602; Jahresber. **1869**, p. 440. Uses phenolsulfonic acid.
- KOPP, E., Ber. **5**, 284 (1872). Used diphenylamine dissolved in  $\text{H}_2\text{SO}_4$  for detecting and estimating nitrites and nitrates. Estimated the nitrate, then oxidized the nitrites to nitrate and estimated total nitrate.
- NICHOLSON, E., Chem. News **25**, 89 (1872); from the Madras monthly J. Med. Sci., May, 1871; cf. Kersting, Ann. Chem. Pharm. **1863**. Uses brucine as recommended by Kersting.
- BLUNT, T. P., Chem. News **25**, 205 (1872); J. Chem. Soc. **25**, 922 (1872); Chem.

- News **26**, 105 (1872). Estimation of nitric acid in potable waters. Note disclaiming to be originator of Nessler's reagent for  $\text{NO}_2$ .
- THORPE, T. E., Proc. Chem. Soc. March 6, 1873; Chem. News **27**, 129 (1873). Uses Cu-Zn couple of Gladstone and Tribe to decompose nitrates with the formation of  $\text{NH}_3$ . Determines  $\text{NH}_3$  with Nessler's reagent if amount is small.
- BOLAS, T., Chem. News **28**, 248, 283 (1873); J. Chem. Soc. **27**, 387 (1874). Testing for nitric acid, and its colorimetric estimation. Uses  $\text{FeSO}_4$  and concentrated  $\text{H}_2\text{SO}_4$ .
- DONKIN, W. F., Proc. Chem. Soc. Nov. 6, 1873; Chem. News **23**, 254 (1873). On the estimation of nitrates in potable waters. Based on the reaction of nitrates in the presence of chlorides, when treated with phenol and  $\text{H}_2\text{SO}_4$ . Gives a reddish solution which changes to blue upon adding an excess of ammonia. Said to detect 1 part in 4,000,000 parts of water. Details must be closely observed.
- BÖTTGER, Jahresber. Tier-Chem., **1875**, p. 918. Proposed using Kopp's [Ber. **5**, 284 (1872)] reaction for the detection of nitrites and nitrates in potable waters.
- KNIGHTS, J. W., Analyst **6**, 56 (1881). Uses brucine in alcoholic solution and  $\text{H}_2\text{C}_2\text{O}_4$  instead of  $\text{H}_2\text{SO}_4$ .
- JOHNSTONE, W., Chem. News **44**, 23 (1881). Says method of Knights is only a slight modification of Nicholson's, Chem. News **25**, 89 (1872) and Madras Monthly J. Med. Sci. May, 1871.
- KNIGHTS, J. W., Chem. News **44**, 46 (1881). Uses the brucine reaction. Claims to be the first to make the reaction quantitative.
- JOHNSTONE, W., Chem. News **44**, 70 (1881). Says Knights was not the first to use the brucine reaction for nitrates and points out that the standard red solution must contain alcohol to prevent fading. Also must be kept from the light.
- KNIGHTS, J. W., Chem. News **44**, 80 (1881). Still claims his brucine method is new and points out the necessity of adding a little  $\text{Ba}(\text{OH})_2$  solution to water containing much sulfate, to prevent free  $\text{H}_2\text{SO}_4$  and consequent partial decomposition of the red coloration.
- GRANDVAL, A. and LAJOUX, H., Compt. rend. **101**, 62 (1885); J. Chem. Soc. **48**, 1093 (1885). Detection and estimation of small quantities of nitric acid in the air, water, soils, etc. Use phenolsulfonic acid.
- HAGER, H., Chem. Zentr. **16**, 586, 588 (1885); J. Soc. Chem. Ind. **4**, 613 (1885). Diphenylamine and crystallized phenol as reagents for nitrates and nitrites.
- SMITH, A. P., Analyst **10**, 199 (1885); *ibid.*, **12**, 50 (1887). Uses phenolsulfonic acid.
- SPIEGEL, L., Ber. **21**, 3568 (1888); briefly described in Z. Hyg. **2**, 189 (1887). Uses diphenylamine.
- FOX, F., Tech. Quart. **1**, 54 (1887-8). Made a few modifications of the Grandval and Lajoux [Compt. rend **101**, 62 (1885)] method.
- LINDO, D., Chem. News **58**, 1, 15, 28 (1888). Phenol and some allied bodies as tests with concentrated sulfuric acid for nitrites, nitrates, and chlorates in aqueous solution.

- HOOKER, S. C., Ber. **21**, 3302 (1888); Analyst **14**, 19 (1889); J. Franklin Inst. **127**, 61. Determination of nitrates in water. Uses carbazol in concentrated  $\text{H}_2\text{SO}_4$  and mentions that probably diphenylamine, etc., in concentrated  $\text{H}_2\text{SO}_4$  might be used instead of carbazol.
- RIDEAL, S., Chem. News **60**, 261 (1889); see also J. Anal. Chem. **4**, 67 (1890); J. Chem. Soc. **58**, 831 (1890). Colorimetric methods for determining nitrates in potable waters. Compares the phenolsulfonic acid and carbazol methods. Results about alike.
- MÜLLER, J. A., Bull. soc. chim. [3], **2**, 670 (1889); J. Chem. Soc. **58**, 415 (1890). Uses diphenylamine.
- HOOKER, S. C., Am. Chem. J. **11**, 249 (1889); Prelim. paper in Ber. **21**, 3302 and J. Franklin Inst. **127**, 61; J. Soc. Chem. Ind. **8**, 138, 569 (1889). A rapid colorimetric method of determining nitrates in potable waters. Uses carbazol in concentrated  $\text{H}_2\text{SO}_4$ . Intense green coloration produced by  $\text{NO}_3$  and other oxidizing agents. Sensitive to 0.0006 mg.  $\text{HNO}_3$ . The usual amount of  $\text{HNO}_2$  in water gives no appreciable error. Fe in amounts greater than 1 part per million must be removed. Chlorides must be removed. Easily destructible organic matter gives low results but unless present in large excess, no serious error is caused.
- MÜLLER, J. A., Chem. News **61**, 100 (1890); Bull. soc. chim.; see also J. Anal. Chem. **4**, 209 (1890). Colorimetric determination of nitric acid by means of a sulfuric acid solution of diphenylamine.
- ROSENFELD, M., Z. anal. Chem. **29**, 661 (1890); J. Chem. Soc. **60**, 496 (1891). Estimation of nitric and nitrous acids in potable waters. Uses pyrogallol.
- JOHNSON, A. E., Chem. News **61**, 15 (1890); see also J. Anal. Chem. **4**, 208 (1890). Uses phenolsulfuric acid containing HCl. Prefers using a standard 1/10 the strength of that used by Rideal, Chem. News **60**, 261 (1889).
- ORMANDY, R. and COHEN, J. B., J. Chem. Soc. **57**, 811 (1890). A new method for the estimation of nitrates and nitrites in water. Use Al-Hg couple and determine  $\text{NH}_3$  by Nessler's reagent.
- HAZEN, A. and CLARK, H. W., Report Mass. Board of Health, **1890**, p. 712; J. Anal. App. Chem. **6**, 5, 301 (1891); J. Am. Chem. Soc. **5**, 301 (1891); Chem. News **64**, 121, 162 (1891); J. Chem. Soc. **62**, 243 (1892). Estimation of nitrates in water. Examine the phenolsulfonic acid method and find it untrustworthy.
- BLAIR, J. A., see "The Organic Analysis of Potable Waters," 2d ed. Appendix D, p. 112, J. A. Churchill, London, **1891**. Reduces to  $\text{HNO}_2$  by  $\text{H}_3\text{AsO}_3$  and  $\text{H}_2\text{SO}_4$  and determines the  $\text{HNO}_2$  (distilled off) by means of metaphenylenediamine.
- HARROW, G., J. Chem. Soc. **59**, 320 (1891); J. Soc. Chem. Ind. **10**, 727 (1891); Chem. News **63**, 223. A rapid method of estimating nitrates in potable waters.  $\text{HNO}_3$  reduced to  $\text{HNO}_2$  by hydrochloric acid and zinc dust in the presence of  $\alpha$ -naphthylamine and  $\text{H}_2\text{SO}_4$ . Pink coloration results.
- BARTRAM, G. H., Chem. News **63**, 228 (1891); J. Franklin Inst. **131**, 385 (1891); J. Soc. Chem. Ind. **10**, 951 (1891). On a source of error in the determination of nitrates in water by the phenolsulfonic acid method.

- MASON, W. P., *Chem. News* **64**, 197 (1891); *J. Chem. Soc.* **62**, 243 (1892). Carbazol method for estimating nitrates in water analysis.
- GILL, A. H., *J. Am. Chem. Soc.* **16**, 122, 193 (1894); *Tech. Quart.* **7**, 55 (1894); *J. Soc. Chem. Ind.* **13**, 663 (1894). Uses phenolsulfonic acid.
- LUNGE, G. and LWOFF, A., *Z. angew. Chem.* **1894**, p. 345; *J. Chem. Soc.* **66**, ii, 398 (1894); *J. Soc. Chem. Ind.* **14**, 67 (1895). Estimation of very small quantities of the nitrogen acids. Use the brucine reaction for  $\text{HNO}_3$ .
- PICHARD, P., *Chem. News* **73**, 2 (1896); from *Compt. rend.* **121**, 758 (1895); *J. Soc. Chem. Ind.* **15**, 829 (1896). Rapid determination of nitric nitrogen in vegetable products. Uses brucine. Concentrated  $\text{H}_2\text{SO}_4$  added in case of nitrates to liberate  $\text{HNO}_3$ .
- ALVAREZ, E. P., *Compt. rend.* **124**, No. 6; *Gazz. chim. ital.* **128** (1897); *Chem. News* **79** (1899). Suggests resorcin and  $\beta$ -naphthol as reagents for  $\text{NO}_2$ ,  $\text{NO}_3$ , and  $\text{ClO}_2$ .
- MOERK, F. X., *Am. J. Pharm.* **71**, 157 (1899); *Analyst* **24**, 222 (1899). A method for facilitating the color comparison in the determination of nitrates in water by the phenolsulfonic acid method.
- RUSSWURM, *Pharm. Zentralhalle* **40**, 516 (1899); *Chem. Zentr.* **2**, 593 (1899); *J. Soc. Chem. Ind.* **18**, 1052 (1899). Uses cresol and concentrated  $\text{H}_2\text{SO}_4$ , finally making the test solution ammoniacal.
- WINKLER, L. N., *Chem.-Ztg.* **23**, 454 (1899); *Chem. News* **81**, 27 (1900). Estimation of ammonia, nitric acid, and nitrous acid in natural waters. Uses Nessler's reagent for  $\text{NH}_3$ , the brucine reaction for  $\text{HNO}_3$ , and a volumetric method (iodine liberated and titrated with  $\text{Na}_2\text{S}_2\text{O}_3$ ) for  $\text{HNO}_2$ .
- CIMMINO, R., *Z. anal. Chem.* **38**, 429 (1899); *J. Soc. Chem. Ind.* **18**, 946 (1899). Uses diphenylamine and  $\text{H}_2\text{SO}_4$  in 5 per cent  $\text{HCl}$  solution.
- KOSTJAMIN, N. N., *Chem.-Ztg. Rep.* **24**, 218 (1900); *J. Soc. Chem. Ind.* **19**, 933 (1900). Uses the brucine reaction.
- MARCILLE, R., *Ann. Agron.* **27**, 596 (1901); *J. Chem. Soc.* **82**, ii, 173 (1902). Estimation of nitrates in chlorinated waters. Adds ammoniacal  $\text{Ag}_2\text{SO}_4$ , evaporates, cools, and then adds phenoldisulfonic acid and finally  $\text{NH}_4\text{OH}$ .
- WINKLER, L. W., *Chem.-Ztg.* **25**, 586 (1901); *J. Soc. Chem. Ind.* **20**, 937 (1901). Uses brucine.
- CAZENEUVE, P. and DEFURNEL, H., *Bull. soc. chim.* **25**, 639 (1901); *Analyst* **26**, 306 (1901); *J. Soc. Chem. Ind.* **20**, 838 (1901). Uses brucine.
- NOLL, H., *Z. angew. Chem.* **14**, 1317 (1901); *J. Chem. Soc.* **82**, ii, 173 (1902). Uses 20 cc.  $\text{H}_2\text{SO}_4$ , sp. gr. 1.84, containing 0.05 g. brucine.
- WINKLER, L. W., *Z. angew. Chem.* **15**, 170; *Analyst* **27**, 162 (1902). The behavior of nitric and nitrous acid with brucine and sulphuric acid.
- MONTANARI, C., *Staz. sper. agrar. ital.* **34**, 690 (1901); *Gazz. chim. ital.* **32**, i, 87 (1902); *J. Chem. Soc.* **82**, ii, 287 (1902). Reaction of the phenolsulfonic reagent in the determination of nitrates by the Granval and Lajoux colorimetric method.
- GEELMUYDEN, H. C., *Z. anal. Chem.* **42**, 276, 518 (1903).
- RICHARDSON, F. W. and HOLLINGS, P., *J. Soc. Chem. Ind.* **22**, 616 (1903). Use phenoldisulfonic acid.



- PAGNOUL, A., Bull. assocn. chim. sucr. dist. **21**, 602 (1903); J. Soc. Chem. Ind. **23**, 135 (1904).
- TATLOCK, R. R. and THOMSON, R. T., J. Soc. Chem. Ind. **23**, 429 (1904). Use phenolsulfonic acid.
- RAIKOW, P., Oesterr. Chem. Ztg. **7**, 557 (1904); Analyst **30**, 174 (1905). The differentiation of nitric and nitrous acid by means of diphenylamine.
- ANDREWS, L. W., J. Am. Chem. Soc. **26**, 388 (1904). Sprengel's method for colorimetric determination of nitrates. Uses phenoldisulfonic acid. Shows that 0.993 g. paranitrophenol per liter (0.1 mg. N per cubic centimeter) can be used as a standard. For use, a measured volume is made alkaline and diluted to match the color obtained in the usual way.
- ALVAREZ, E. P., Bull. soc. chim. **33**, 717 (1905); Analyst **30**, 285 (1905). Observations on the use of diphenylamine as a reagent for nitrites, nitrates, and chlorates.
- ALVAREZ, E. P., Chem. News **91**, 155 (1905). Observations on diphenylamine as reagent for nitrites, nitrates, chlorates, and its use when mixed with resorcin and  $\beta$ -naphthol. Use diphenylamine and resorcin for  $\text{NO}_2$  and  $\text{NO}_3$  and diphenylamine and  $\beta$ -naphthol for  $\text{ClO}_3$ .
- FRERICHS, G., Arch. Pharm. **243**, 80 (1905); Chem.-Ztg. Rep. **29**, 84 (1905); Analyst **30**, 174 (1905); J. Soc. Chem. Ind. **24**, 348 (1905).
- BAY, I., Compt. rend. **140**, 796 (1905); Chem. News **91**, 190 (1905). Action of diphenylamine on nitric acid.
- HINRICHS, C. G., Bull. soc. chim. **33**, 1002 (1905); Analyst **30**, 381 (1905). The differentiation of nitrates from other oxidizing agents by the diphenylamine reaction.
- STEWART, R. and GREAVES, J. E., Utah Expt. Sta. Bull. **106** (1906).
- SYME, W. A., J. Ind. Eng. Chem. **1**, 188 (1909); C. A. **3**, 1134 (1909); J. Soc. Chem. Ind. **28**, 375 (1909). The colorimetric determination of nitrates in soil solutions containing organic matter. Phenoldisulfonic and  $\text{NH}_4\text{OH}$  method is inaccurate if chlorides, nitrites, or organic matter are present.
- PERRIER, G. and FARCY, L., Bull. soc. chim. [iv], **5**, 178 (1909); Analyst **31**, 174 (1909). The influence of chlorides on the estimation of nitrates. Use method of Grandval and Lajoux.
- FARCY, L., Bull. soc. chim. [iv], **5**, 563 (1909); Analyst **34**, 335 (1909). The influence of bromides and iodides on the estimation of nitrates in water. Uses method of Grandval and Lajoux.
- MARCILLE, R., Ann. chim. anal. **14**, 303 (1909); J. Soc. Chem. Ind. **28**, 957 (1909). Removes chlorides by adding  $\text{Ag}_2\text{SO}_4$ . Then uses the Grandval and Lajoux method.
- FARCY, L., Bull. soc. chim. **5**, 775 (1909); J. Soc. Chem. Ind. **28**, 849 (1909). Compares several methods. F's modified Grandval and Lajoux method preferred in presence of chlorides and  $\text{NH}_4$  salts.
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- CHAMOT, E. M. and PRATT, D. S., J. Am. Chem. Soc. **31**, 922 (1909). A study of the phenolsulfonic acid method for the determination of nitrates in water.
- I. The composition of the reagent and the reaction product. An important paper. Evidence is presented to show that the yellow color produced in the cold is due to the nitration of phenol-2 : 4-disulfonic acid, the compound formed being the alkali derivative of a nitrophenolsulfonic acid. Paper contains a bibliography of 48 references (discussed in the text) arranged (approximately) chronologically. Many of the references are on the sulfonic acids of phenol.
- FARCY, L., Bull. soc. chim. **5**, 1090 (1909). Influence of nitrites on determination of nitrates by the Grandval-Lajoux process. "A trace of nitrates added to a mixture of nitrites and chlorides gives a more intense color than the amount of nitrates added should give by the Grandval-Lajoux method. This is explained by the liberation of Cl, which oxidizes some of the nitrites in nitrates. It is, therefore, necessary in detg. nitrates by the Grandval-Lejoux method in the presence of nitrite first to determine the nitrites and then remove them either by oxidizing with  $\text{KMnO}_4$  or destroying by means of urea."
- G. B. Frankforter, C. A. **4**, 802 (1910).
- LOMBARD, M. and LAFORE, J., Bull. soc. chim. [iv], **5**, 321 (1909). Remarks on the determination of nitrates by the method of Grandval and Lajoux.
- FARCY, L., Bull. soc. chim. [iv], **5**, 1088 (1909); Analyst **35**, 81 (1910); J. Soc. Chem. Ind. **29**, 107 (1910). Estimation of nitrates by the method of Grandval and Lajoux in waters containing chlorides or nitrites.
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- TILLMANS, J., Z. Nahr. Genussm. **20**, 676 (1910); J. Soc. Chem. Ind. **30**, 44 (1911). Detection and estimation of nitric acid in milk by diphenylamine-sulfuric acid.
- STEWART, R. and GREAVES, J. E., J. Am. Chem. Soc. **32**, 756 (1910); see also Utah Expt. Sta. Bull. **106**, 80 (1906). The influence of different quantities of chlorides on the phenyldisulphonic acid method for nitrates. Results

- show that chlorine, even when present in amounts as low as 2.6 parts per million, affects the results for nitric nitrogen by this method.
- CHAMOT, E. M. and PRATT, D. S., *J. Am. Chem. Soc.* **32**, 630 (1910). A study of the phenolsulfonic acid method for the determination of nitrates in water. II. The composition of the yellow compound. Find that the coloration is due to the formation of tripotassium 6-nitrophenol-2 : 4-disulfonate.
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- CHAMOT, E. M., PRATT, D. S. and REDFIELD, H. W., *J. Am. Chem. Soc.* **33**, 381 (1911). A study of the phenoldisulfonic acid method for the determination of nitrates in water. IV. A modified phenoldisulfonic acid method.
- ROMIJN, G., *Z. anal. Chem.* **50**, 566 (1911); *Analyst* **36**, 467 (1911); *J. Soc. Chem. Ind.* **30**, 1009 (1911).
- CARON, H., *Ann. chim. anal.* **16**, 211 (1911); *J. Chem. Soc.* **100**, ii, 767 (1911); *J. Soc. Chem. Ind.* **30**, 837 (1911); *Z. anal. Chem.* **52**, 379 (1913). Uses diphenylamine.
- DENIGÈS, G., *Bull. soc. chim. [iv]*, **9**, 544 (1911); *J. Chem. Soc.* **100**, ii, 655 (1911); *J. Soc. Chem. Ind.* **30**, 827 (1911). Detection of nitrates and nitrites in water by means of reduced strychnine. Uses a reagent prepared by adding 5 grams of Zn amalgam to a mixture of 5 cc. HCl (sp. gr. 1.18) with 5 cc. of a 1 per cent solution of strychnine sulfate. Boil, cool, and decant. 10 cc. water containing 0.0001 g.  $\text{HNO}_2$  per liter gives a red coloration with 0.5 cc. of the reagent. Nitrates give a color only in the presence of  $\text{H}_2\text{SO}_4$ .
- TILLMANS, J., and SPLITTGERBER, A., *Z. Nahr. Genussm.* **22**, 401 (1911). Estimation of nitric acid in milk by means of diphenylamine-sulfuric acid.
- JOHNSON, A. E., *Chem. News* **104**, 235 (1911); *J. Soc. Chem. Ind.* **30**, 1404 (1911). Uses phenolsulfonic acid.
- WITHERS, W. A. and RAY, B. J., *J. Am. Chem. Soc.* **33**, 708 (1911); *J. Chem. Soc.* **100**, ii, 656 (1911); *J. Soc. Chem. Ind.* **30**, 708 (1911). Modification of the diphenylamine test for nitrous and nitric acids. One part of nitrous nitrogen can be detected in 25 million, or one part of nitric nitrogen in 35 million by heating 15 to 20 minutes. Heating one hour increases the sensitivity to 1 part in 32 million and 1 part in 44 million, respectively.
- VERMEHREN, A., *Centr. Zuckerind.* **19**, 72; *C. A.* **5**, 55 (1911). The determination and estimation of small quantities of nitric and nitrous acid in water.
- TILLMANS, J. and SUTTHOFF, W., *Z. anal. Chem.* **50**, 473 (1911); *C. A.* **5**, 3211 (1911); *J. Soc. Chem. Ind.* **30**, 918 (1911). A simple method for the determination and estimation of nitric and nitrous acids in water. By adding NaCl as reagent to the reacting mixture (diphenylamine reaction) the degree of accuracy uniformly obtainable may be increased to 0.1 mg.  $\text{HNO}_2$  per liter. Nitrites if present react similarly to nitrates and a combined determination may be made. Give a long list of references on the diphenylamine-reaction with nitrates.

- DENIGÈS, G., *Compt. rend.* **152**, June 19 (1911); *Chem. News* **104**, 119 (1911). Uses strychnine reagent.
- CARON, H., *Répert. pharm.* [3] **23**, 385; *Ann. chim. anal.* **17**, 9; cf. *C. A.* **5**, 1379 (1911); *C. A.* **6**, 2246 (1912). Determination of nitrates in urine. Uses diphenylamine. Results only approximate, but method is easy, rapid and requires only a few cubic centimeters of urine.
- SILVESTER, H., *J. Soc. Chem. Ind.* **31**, 95 (1912); *J. Chem. Soc.* **102**, ii, 386 (1912). The phenolsulfonic acid method of estimating nitrates in sewage effluents. Points out possibility of error due to thiocyanates which may be present.
- GRÜNHUT, L., *Z. anal. Chem.* **51**, 519 (1912). Test and determination of nitrates in milk. A review.
- TILLMANS, J. and SPLITTGERBER, A., *Z. Nahr. Genussm.* **23**, 49 (1912); *Analyst* **37**, 140 (1912). Estimation of potassium nitrate in meats. When not over 1.5 per cent  $\text{KNO}_3$ , use Noll's brucine-sulfuric acid method or the diphenylamine-sulfuric method.
- LIPMAN, C. B. and SHARP, L. T., *Biedermann's Zentr.* **42**, 721 (1913); *Univ. Calif. Pub. Agr. Sci.* **1**, 21 (1912); *J. Chem. Soc.* **106**, ii, 145 (1914). Phenoldisulfonic acid method for estimating nitrates in soils.
- SILBER, J., *Z. Nahr. Genussm.* **26**, 282 (1913); *J. Chem. Soc.* **104**, ii, 978 (1913). Uses the phenolsulfonic acid method of Grandval and Lajoux.
- STEWART, R. and GREAVES, J. E., *Chem. News* **108**, 97 (1913) and *J. Am. Chem. Soc.* **35**, 579 (1913). The influence of chlorine on the determination of nitrates by the phenoldisulfonic acid method.
- SILBER, J., *Z. Nahr. Genussm.* **26**, 282 (1913). Colorimeter for the determination of nitrates in water.
- KELLEY, W. P., *Chem. News* **108**, 178 (1913) and *J. Am. Chem. Soc.* **35**, 775 (1913). The effect of sulfates on the determination of nitrates.
- ELSDON, G. D. and SUTCLIFFE, J. A. L., *Analyst* **38**, 450 (1913). Nitrates and nitrites in milk. Uses the brucine-sulphuric acid method for nitrates and the Griess-Ilosvay method for nitrites.
- AUTENRIETH, W. and FUNK, A. *Z. anal. Chem.* **52**, 137 (1913); *C. A.* **7**, 3627 (1913). Colorimetric methods for water analysis by use of the Autenrieth-Koenigsberger colorimeter. Details are given for the estimation of  $\text{NH}_4$ ,  $\text{HNO}_2$ ,  $\text{HNO}_3$ , Fe, Pb, and  $\text{H}_2\text{S}$ .
- LETTIS, E. A. and REA, F. W., *J. Chem. Soc.* **105**, 1157 (1914); *Proc. Chem. Soc.* **30**, 72; *C. A.* **8**, 2323 (1914); *J. Soc. Chem. Ind.* **33**, 570 (1914). An extremely delicate colorimetric method for detecting and estimating nitrates and nitrites. Diphenylbenzidine used for nitrates. Nitrites oxidized by  $\text{KMnO}_4$  and similarly determined.
- DENIGÈS, G., *Ann. chim. anal.* **19**, 221 (1914); from *Bull. soc. pharm. Bordeaux*, 1914. Preparation of reduced strychnine reagent for the colorimetric estimation of nitrates in water.
- WINKLER, L. W., *Z. Nahr. Genussm.* **29**, 10 (1915); *J. Chem. Soc.* **110**, ii, 490 (1916). Detection and estimation of nitrates in waters. Uses starch solution, KI, and  $\text{H}_3\text{PO}_4$ .

- ALLEN, E. R., *Chem. News* **112**, 269 (1915); from *J. Ind. Eng. Chem.* **7**, 524 (1915). Uses diphenylamine-sulfuric acid as modified by Withers and Ray, *J. Am. Chem. Soc.* **33**, 708 (1911), except that tests were made at room temperature instead of at 40°. One part of nitric nitrogen in 25,000,000 parts of solution could be detected.
- POTTER, R. S. and SNYDER, R. S., *J. Ind. Eng. Chem.* **7**, 863 (1915). The determination of nitrates in soil. Use the phenoldisulfonic acid method as modified by Chamot and collaborators.
- ROMIJN, G., *Chem. Weekblad* **12**, 23 (1915); *Chem. Zentr.* 764 (1915).
- ARNY, H. V. and RING, C. H., *J. Ind. Eng. Chem.* **8**, 309 (1916); *C. A.* **10**, 1146 (1916); see also *Proc. 8th Intern. Cong. Appl. Chem.* **26**, 319; cf. Arny and Pickhardt, *Drug. Circ.* **58**, 131 (1914) and *J. Franklin Inst.* Aug. 1915. Color standards and colorimetric assays. Use colored solutions of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to prepare standard color solutions. Phenolsulfonic acid method used for  $\text{NO}_3$ .
- POTTER, R. S. and SNYDER, R. S., *J. Am. Soc. Agron.* **8**, 54 (1916). The determination of nitrates in soil.
- ACÉL, D., *Z. Nahr. Genussm.* **31**, 332 (1916); *C. A.* **11**, 1493 (1917). Detection and quantitative determination of nitrates and nitrites in meats and sausages. The reaction between  $\text{HNO}_2$  and a solution of  $\alpha$ -naphthylaminesulfanilic acid containing  $\text{AcOH}$  gives a red color which may be compared with standard solutions of fuchsin. Gives procedures both in presence and absence of nitrate.
- KOLTHOFF, I. M., *Utrecht. Pharm. Weekblad* **54**, 633; *C. A.* **11**, 2701 (1917). Chemical study of potable waters. III. Ammonia, nitrite and nitrate.
- SMITH, L., *Z. anal. Chem.* **56**, 28 (1917); *J. Chem. Soc.* **112**, ii, 217; *C. A.* **11**, 2759 (1917). For determining  $\text{HNO}_3$ , diphenylbenzidine is about twice as sensitive as diphenylamine.
- DAVIS, C. W., *J. Ind. Eng. Chem.* **9**, 290 (1917); *J. Chem. Soc.* **112**, ii, 329 (1917). The phenoldisulfonic acid method for estimating nitrates in soils.
- TILLMANS, J., *Z. anal. Chem.* **56**, 509 (1917); *J. Soc. Chem. Ind.* **37**, 163A; *C. A.* **12**, 1865 (1918). See also *Z. Nahr. Genussm.* **22**, 401 (1911) and **20**, 676 (1910). Use of diphenylamine-sulfuric acid for colorimetric determinations, e. g., of nitrates of milk. A reply to L. Smith (*Z. anal. Chem.* **56**, 28). Says method is trustworthy if test solution and standard are treated in exactly the same way.
- PHELPS, E. B., and SHOUB, H. L., *J. Ind. Eng. Chem.* **9**, 767 (1917); *J. Chem. Soc.* **112**, ii, 504 (1917). Estimation of nitrates in sewage by means of *o*-toluidine.
- GERICKE, W. F., *J. Ind. Eng. Chem.* **9**, 585 (1917); *J. Chem. Soc.* **112**, ii, 421 (1917); *J. Soc. Chem. Ind.* **36**, 904 (1917). Estimation of nitrate in the presence of chlorides. Uses phenoldisulfonic acid reagent.
- RICHARDSON, F. W., *J. Soc. Chem. Ind.* **33**, 13 (1917); *C. A.* **11**, 1383 (1917). Estimation of free sulphuric, nitric, and picric acids in the presence of each other. Uses phenoldisulfonic acid for  $\text{HNO}_3$ .

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- OWLSNER, ALICE, *Z. angew. Chem.* **31**, 170, 178 (1918); *J. Chem. Soc.* **114**, ii, 405 (1918). A survey of methods for the estimation of nitrites and nitrates in the same solution.
- NOYES, H. A., *J. Ind. Eng. Chem.* **11**, 213 (1919); *J. Soc. Chem. Ind.* **38**, 265A (1919). Estimation of nitrates in soil by the phenol-disulfonic acid method.
- FREDERICK, R. C., *Analyst* **44**, 281 (1919); *J. Chem. Soc.* **116**, ii, 371 (1919); *Chem. News* **118**, 298 (1919); *C. A.* **13**, 3259 (1919); *J. Soc. Chem. Ind.* **38**, 694A (1919). Uses phenoldisulfonic acid.
- HAUN, F., *Z. Nahr. Genussm.* **39**, 355 (1920); *Analyst* **45**, 382 (1920). Preparation of diphenylamine-sulphuric acid reagent. Heats the  $\text{H}_2\text{SO}_4$  to reduce any trace of  $\text{Fe}^{+++}$  to  $\text{Fe}^{++}$ . Ferrous salts do not interfere with the  $\text{HNO}_3$  test, but ferric salts cause a blue coloration in the diphenylamine reagent.
- KOLTHOFF, I. M., *Pharm. Weekblad* **57**, 1253 (1920); *C. A.* **15**, 352 (1921); *J. Soc. Chem. Ind.* **39**, 761A (1920). Colorimetric determination of ammonia, nitrite, and nitrate. Studies were made of the influence of time, temperature, amount of reagent and presence of impurities on the accuracy of colorimetric determinations.
- SNELL, F. D., *Colorimetric Analysis*, p. 108, D. Van Nostrand Co., New York, 1921. Determination of nitric acid by brucine.
- SNELL, F. D., *ibid.*, p. 110, 1921. Determination of nitric acid by diphenylbenzidine.
- SNELL, F. D., *ibid.*, p. 111, 1921. Determination of nitric acid by phenolsulfonic acid.
- SNELL, F. D., *ibid.*, p. 113, 1921. Determination of nitric acid by pyrogallol.
- LAMPE, B., *Wochschr. Brau.* **39**, 303 (1922); *C. A.* **17**, 3066 (1923); *J. Soc. Chem. Ind.* **42**, 198A (1923). Uses a simple modification of the Grandval and Lajoux method.
- GIMINGHAM, C. T. and CARTER, R. H., *J. Agr. Sci.* **13**, 60 (1923); *J. Chem. Soc.* **124**, ii, 577 (1923). Estimation of nitrates in soils by the phenoldisulfonic acid method.
- NIXON, I. G., *Chem. News* **126**, 261 (1923); *Chem. Zentr.* **94**, IV, 226 (1923). A new test for nitrate and nitrite. Uses "G Salt" (2 : 6 : 8 naphthol disulfonic acid). A distinct red color is given by 0.02 mg. N as  $\text{HNO}_3$ , while with much smaller quantities a faint yellow color is obtained. Application of moderate heat often deepens the color. To make the test: 1 cc. of sample is mixed with 1 cc. concentrated  $\text{H}_2\text{SO}_4$  and then 5 cc. of 1 per cent solution "G Salt" added slowly. If nitrate or nitrite is present an intense wine red color develops.
- SCALES, F. M. and HARRISON, A. P., *Ind. Eng. Chem.* **16**, 571 (1924); *C. A.* **18**, 1960 (1924); *Analyst* **49**, 405 (1924). Based upon Denigès, *Bull. soc. chim.* [4], **9**, 544 (1911). Mix equal volumes of 0.5 per cent strychnine sulfate in concentrated  $\text{HCl}$  and 0.1 per cent  $\text{HgCl}_2$  in pure water.
- HARPER, H. J., *Ind. Eng. Chem.* **16**, 180 (1924); *J. Soc. Chem. Ind.* **43**, B268

- (1924). The accurate determination of nitrates in soils. Uses phenoldisulfonic acid.
- SHMUK, A., *Nauk. Agron. Zhur.* **1**, 562 (1924); *Expt. Sta. Record* **54**, 111; *C. A.* **20**, 3470 (1926). Determination of nitrates in fresh plant materials. Uses sulfophenol.
- KOLTHOFF, I. M., *Chem. Weekblad* **21**, 423 (1924); *J. Chem. Soc.* **126**, ii, 779 (1924). The hydrostrychnine reagent for nitrites and nitrates. "Denigès' method for prep. the reagent (*C. A.* **5**, 3712) is modified by replacing amalgamated Zn with pure Zn. A piece of amalgamated Zn increases the stability of the reagent which is to be used within a short time. Blank tests are necessary. Scales' and Harrison's modification (*C. A.* **18**, 1960) using Mg instead of Zn yields inconsistent results. The reaction is carried out as recommended by Denigès, the max. coloration occurring after 5-10 min. standing. The reagent gives a red or pink coloration with nitrites, chromates, halogenates and ferricyanides in the presence and absence of concd.  $\text{H}_2\text{SO}_4$ , with nitrates and  $\text{Fe}^{+++}$  only in the presence of the latter." Mary Jacobsen, *C. A.* **19**, 222 (1925).
- EKKERT, L., *Pharm. Zentr.* **66**, 649 (1925); *J. Chem. Soc.* **128**, ii, 1093 (1925). The diphenylamine reaction for nitrates. Points out the reaction is not specific for  $\text{HNO}_3$ , but arises from the presence of many other oxidizing agents. Tabulates the common oxidizing agents that produce a coloration.
- HIBBARD, P. L., *Ind. Eng. Chem.* **17**, 58 (1925). Modification of Scales' method for determination of nitrates. Scales' method is satisfactory only if any  $\text{Na}_2\text{CO}_3$  or  $\text{NaOH}$  present in the  $\text{MgO}$  is neutralized.
- VÁGI, S., *Z. anal. Chem.* **66**, 14 (1925). New reactions of nitrate and nitrite. "A soln. of 1-2 g. benzidine in 100 cc. of 50%  $\text{AcOH}$  dild. with 300 cc. of water reacts neither with nitrates nor chlorates but gives an intense yellow color with nitrite; 0.05 mg. of  $\text{N}_2\text{O}_5$  per liter suffices to give the test but 100-200 cc. of the aq. soln. of nitrite and 10 cc. of the reagent must be used then. Nitrites, chlorates,  $\text{FeCl}_3$  and other oxidizers react with Na 1, 8-dihydroxy-3, 6-naphthalenedisulfonate, 1, 5-naphtholsulfonic acid and Na 1, 8-aminonaphtholdisulfonate, which give colorations with nitrate. For the  $\text{HNO}_3$  test, 5 cc. of a nitrate soln., 5 cc. of concd.  $\text{H}_2\text{SO}_4$  and a drop of the aq. soln. of these last 3 org. substances were used. The presence of 0.15 g.  $\text{KNO}_3$  per l. could be detected. The first and last of the 3 compds. give a red or orange color and the other gives a wine red." W. T. H., *C. A.* **19**, 2000 (1925).
- VÁGI, S., *Z. anal. Chem.* **66**, 101 (1925); cf. *C. A.* **19**, 2000 (1925). New reactions for nitrate and nitrite. II. "Pyrogallol or pyrocatechol can be used for the colorimetric detn. of 0.5-1 mg. of nitrite. Five cc. of a 1% soln. in 50%  $\text{AcOH}$  should be used for 40 cc. of aq. soln.; reddish brown colorations are obtained which become yellowish on standing when pyrogallol is used. Nitrates give no color. Hydroquinol in 50%  $\text{AcOH}$  gives an intense yellow coloration with nitrite but will not detect less than 1 mg. of  $\text{N}_2\text{O}_5$  per l. One g. of orcinol dissolved in 300 cc. of 50%  $\text{AcOH}$  is a good reagent for detecting 1-5 mg. of  $\text{N}_2\text{O}_5$  per l.: an intense yellow coloration is produced. One g. of

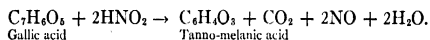
- resorcinol dissolved in 400 cc. of 25% AcOH gives a yellow color with nitrite and is suitable for the colorimetric detn. of 1-5 mg. of  $N_2O_3$  per l. L. Ilosvay recommended a soln. of sulfanilic acid and  $\alpha$ -naphthylamine as reagent for nitrite. Successful attempts were made to replace the latter constituent of the reagent. One g. of  $\alpha$ -naphthol and 0.5 g. of sulfanilic acid dissolved in 100 cc. of AcOH and diluted with 300 cc. of water is a sensitive reagent for  $N_2O_3$ ; a yellow-orange color is obtained, and the test is good for 0.0-1 mg. per l.  $\beta$ -Naphthol proved less satisfactory but nitrite can be used to distinguish between  $\alpha$ - and  $\beta$ -naphthol as the tint is different in the test. A sensitive reagent for nitrite is obtained by dissolving 1 g. of pyrocatechol and 0.5 g. of sulfanilic acid in 100 cc. of AcOH and dilg. with 300 cc. of water. A pink color is obtained with 5 cc. of water contg. 0.1-1 mg.  $N_2O_3$  per l. Nitrite and nitrate both give green colorations with 1% pyrocatechol or pyrogallol in concd.  $H_2SO_4$ . A soln. of 1 g. benzidine and 1 g. of Na aminonaphtholdisulfonate (1, 8, 3,6) in 100 cc. of 80% AcOH and diluted with 300 cc. of water is a good reagent for 0.1-5 mg.  $N_2O_3$ . Five cc. to 40 cc. of nitrite soln. should be used; a red coloration is obtained." W. T. H., C. A. **19**, 2463 (1925).
- SCOTT, W. W., Standard Methods of Chemical Analysis, 4 ed., p. 1416, D. Van Nostrand Co., New York, 1925. Determination of nitrates in water. Reduces with Al foil and Nesslerizes.
- Standard Methods for the Examination of Water and Sewage, 6 ed., pp. 19-22, American Public Health Association, New York, 1925. Uses the phenoldisulfonic acid and the reduction (with Al foil) methods.
- HAASE, L. W., Chem.-Ztg. **50**, 372 (1926); C. A. **20**, 2472 (1926). Uses 5 per cent solution of brucine in pure  $CHCl_3$ . Colors produced are intense; they do not change on standing 24 hours and good results are obtained with solutions containing 0.5 to 20 mg. of  $N_2O_3$  per liter. Stronger solutions must be diluted.

#### Nitrite (and Nitrous Acid).

- HOLLAND, P., Chem. News **17**, 123 (1868). Adds KI, then dilute  $H_2SO_4$  and matches the resulting iodine solution.
- GRIESS, P., Ann. Chem. u. Pharm. **154**, 333; Z. anal. Chem. **10**, 92 (1871). Used (1 : 3.5) diamidobenzoic acid.
- KOPP, E., Ber. **5**, 284 (1872). Uses diphenylamine dissolved in  $H_2SO_4$  for detecting and estimating nitrites and nitrous acid in commercial sulfuric acid.
- FISCHER, F., Dingler's polytech. J. **212**, 404 (1874); J. Chem. Soc. **28**, 185 (1875). Estimation of nitrous acid in potable waters. Reviews several methods (mostly colorimetric).
- NICHOLSON, E., Chem. News **32**, 163 (1875); J. Chem. Soc. **29**, 744 (1876). On the estimation of nitrites in water. Prefers the reaction with KI to that with permanganate. Also mentions P. Holland's colorimetric process [Chem. News **17**, 123 (1868)].
- NICHOLSON, E., Am. Chemist **6**, 297 (1875); from Chem. News **32**, 163 (1875). The estimation of nitrites in water. Reviews several methods.
- BÖTTGER, Jahresber. Tier-Chem., **1875**, p. 918. Proposed using Kopp's [Ber.



- 5**, 284 (1872)] reaction for the detection of nitrites and nitrates in potable waters.
- GRIESS, P., Ber. **11**, 624 (1878); J. Chem. Soc. **34**, 605 (1878); Z. anal. Chem. **17**, 369 (1878); *ibid.*, **18**, 127 (1879). Finds that metaphenylenediamine (m.p. 63°) may with advantage be substituted for diamidobenzoic acid. The development of the yellow color with nitrous acid is due to the formation of triamidoazobenzene:  $2C_6H_5N_2 + HNO_2 \rightarrow C_{12}H_{12}N_6 + 2H_2O$ .
- PREUSSE, C. and TIEMANN, F., Ber. **11**, 627 (1878); J. Chem. Soc. **34**, 606 (1878). Colorimetric with metaphenylenediamine as proposed by Griess.
- LEEDS, A. R., J. Am. Chem. Soc. **1**, 136 (1879); Z. anal. Chem. **18**, 535 (1879); Chem. News **40**, 38, 61 (1879); J. Chem. Soc. **36**, 964 (1879). Detection and estimation of nitrous acid in potable waters. Discusses colorimetric methods with (1) metadiamidobenzene (Griess) and (2) KI (Trommsdorf).
- GRIESS, P., Ber. **12**, 426 (1879).
- WARINGTON, R., J. Chem. Soc. **39**, 231 (1881). Uses naphthylamine.
- WILLIAMS, M. W., Analyst **6**, 36 (1881). Uses metaphenylenediamine.
- SMITH, A. P., Analyst **7**, 65 (1882). On metaphenylenediamine as a reagent for the determination of nitrites in water.
- DAVY, E. W., Chem. News **46**, 1 (1882); J. Chem. Soc. **42**, 1317 (1882). New and expeditious method for the determination of nitrites under various circumstances. Uses gallic acid and little HCl or  $H_2SO_4$ . Exact limit not determined. Delicate to at least 1 part of nitrous acid in 20,000,000 parts of water. Method based on the oxidation of gallic acid by  $HNO_2$ .
- DAVY, E. W., Pharm. J. Trans. [3], **13**, 466; J. Chem. Soc. **44**, 515 (1883). Method based on the reaction:



- HAGER, H., Chem. Zentr. **16**, 586, 588; J. Soc. Chem. Ind. **4**, 613 (1885). Diphenylamine and crystallized phenol as reagents for nitrates and nitrites.
- WARINGTON, R., Chem. News **51**, 39 (1885). On the various methods of detecting nitrous acid. Shows Griess' naphthylamine test the most delicate of all.
- SMITH, A. P., Analyst **12**, 50 (1887). Uses naphthylamine.
- SMITH, A. P., Analyst **12**, 152 (1887). Proves the accuracy of the naphthylamine test.
- ZAMBELLI, L., Chem. Zentr., **1887**, p. 45; J. Chem. Soc. **52**, 533 (1887). Colorimetric determination of nitrites in water. Uses sulphanilic acid and  $\alpha$ -naphthol; also replaces  $\alpha$ -naphthol with phenol.
- LINDO, D., Chem. News **58**, 1, 15, 28 (1888). Phenol and some allied bodies as tests (with concentrated sulphuric acid) for nitrites, nitrates, and chlorates in aqueous solution.
- LUNGE, G., Z. angew. Chem. No. 23 (1889); Analyst **15**, 17 (1890). Uses sulphanilic acid and naphthylamine.
- ILOSVAY, L., Bull. soc. chem. [3], **2**, 388 (1889). L'acide azoteux dans la salive et dans l'air exhale.

- WURSTER, C., Ber. **22**, 1909 (1889); J. Anal. Chem. **3**, 424 (1889). An addition of  $\text{NH}_4\text{Ac}$  to the water to be tested for nitrites according to Griess' methods (Ber. **11**, 624; **12**, 427) causes a much more rapid development of the color.
- THRESH, J. C., Chem. News **62**, 203; from the Pharmaceutical Era, Sept. 20, 1890; J. Anal. Chem. **4**, 455 (1890). The estimation of nitrites in potable waters. Uses KI and starch solution.
- ROSENFELD, M., Z. anal. Chem. **29**, 661 (1890); J. Chem. Soc. **60**, 496 (1891). Estimation of nitric and nitrous acids in potable waters. Uses pyrogallol.
- ORMANDY, R. and COHEN, J. B., J. Chem. Soc. **57**, 811 (1890). A new method for the estimation of nitrates and nitrites in water. Use  $\text{Al-Hg}$  couple and determine  $\text{NH}_3$  by Nessler's reagent.
- VAN DEVENTER, C. M. and JÜRGENS, B. H., Ber. **26**, 932 (1893); cf. Ber. **26**, 589; J. Chem. Soc. **64**, ii, 298 (1893). Investigation of potable water by Schaffer's nitrite reaction.
- ILOSVAY, L., Z. anal. Chem. **33**, 223 (1894); cf. Z. anal. Chem. **56**, 101 (1925). A solution of sulfanilic acid and  $\alpha$ -naphthylamine in acetic acid.
- LUNGE, G. and LWOFF, A., Z. angew. Chem. **12**, 345 (1894); J. Soc. Chem. Ind. **14**, 67 (1895); J. Chem. Soc. **66**, ii, 398 (1894). Use the brucine reaction for  $\text{HNO}_3$  and  $\alpha$ -naphthylamine and sulfanilic acid.
- WILEY, "Agricultural Analysis," **1894**, p. 567. Uses the coloration produced with a solution of ferrous salt. Picini's method.
- DENIGÈS, G., J. Pharm. [6] **2**, 289 (1895); J. Chem. Soc. **70**, 336 (1896). Describes three reagents for nitrites: (1) two solutions, *a*, phenol,  $\text{H}_2\text{SO}_4$  and water; *b*,  $\text{HgAc}$ ,  $\text{H}_2\text{SO}_4$  and water; (2) aniline and glacial  $\text{HAc}$  in water; (3) resorcinol and  $\text{H}_2\text{SO}_4$ .
- GILL, A. H. and RICHARDSON, H. A., J. Am. Chem. Soc. **18**, 21 (1896); J. Chem. Soc. **70**, 340 (1896); J. Soc. Chem. Ind. **15**, 220 (1896). Estimation of nitrites in potable waters. Recommend removing peaty matter with an emulsion of alumina when testing peaty waters by Trommsdorff's iodo-zinc starch test, or by Griess'  $\alpha$ -naphthylamine reaction.
- SCHUYTEN, M. C., Chem.-Ztg. **20**, 722 (1896); J. Chem. Soc. **72**, ii, 596 (1897); J. Soc. Chem. Ind. **15**, 743 (1896). A new reagent for detecting and estimating nitrites. Ten per cent solution of antipyrin in  $\text{HAc}$ . Green coloration produced by nitrites. Not interfered with by heavy metals or organic matter. Delicate to 1 in 20,000 or better.  $\text{F}^{+++}$  salts, free  $\text{HCl}$ , and  $\text{H}_2\text{SO}_4$  interfere.
- ZAMBELLI, L., Chem. Zentr., **1896**, I, 1283; Mon. sci. (IV) **10**, 351; J. Chem. Soc. **72**, 343 (1897); J. Soc. Chem. Ind. **15**, 617 (1896). Estimation of very small quantities of nitrous acid. Reagent prepared by dissolving 2 g. sulphanilide and 2 g. phenol in 25 cc.  $\text{H}_2\text{SO}_4$  and mixed with 25 cc. water.
- PICHARD, P., Compt. rend. **123**, 590; J. Soc. Chem. Ind. **15**, 829 (1896). Detection of nitrites in the presence of sulfites.
- BARBET and JANDRIER, J. pharm. chim. [6] **4**, 248 (1896); J. Chem. Soc. **72**, ii, 234 (1897). Estimation of nitrites in waters. Propose resorcinol as a substitute for metaphenylenediamine.

- RIEGLER, E., *Z. anal. Chem.* **36**, 306 (1897); *J. Chem. Soc.* **72**, ii, 385 (1897); *J. Soc. Chem. Ind.* **16**, 638 (1897). Uses 0.05 g. crystallized naphthionic acid and 5-6 drops concentrated HCl, shakes and adds 3 drops concentrated  $\text{NH}_4\text{OH}$ . Rose-red color produced. Sensitive to 0.01 mg.  $\text{N}_2\text{O}_3$  in 100 cc.
- RIEGLER, E., *Z. anal. Chem.* **36**, 377 (1897); *J. Chem. Soc.* **72**, ii, 464 (1897); *J. Soc. Chem. Ind.* **16**, 699 (1897). Extremely sensitive reagent for detection and colorimetric estimation of nitrous acid. Solution made of 2 g. of pure sodium 1 : 4-naphthylaminesulfonate and 1 g.  $\beta$ -naphthol in 200 cc. and filtered.
- KÖNIG, F. J., *Chem.-Ztg.* **21**, 599 (1897); *J. Chem. Soc.* **74**, ii, 313 (1898); *J. Soc. Chem. Ind.* **16**, 936 (1897).
- ALVAREZ, E. P., *Compt. rend.* **124**, No. 6 (1897); *Gazz. chim. ital.* **128** (1897); *Chem. News* **79** (1899). Suggests recorsin and  $\beta$ -naphthol as reagents for  $\text{NO}_2$ ,  $\text{NO}_3$ , and  $\text{ClO}_3$ .
- PAEPE, D. DE, *Bull. Assocn. Belge des Chim.* **12**, 98; *J. Soc. Chem. Ind.* **17**, 875 (1898). Estimation of nitrites in water. Examines various colorimetric methods and gives preference to the following: 1. Zambelli's reaction:  $\alpha$ -naphthol sulfanilic acid in alkaline solution. 2. Riegler's reaction:  $\beta$ -naphthol naphthonic acid in ammoniacal solution. 3. Griess' reaction:  $\alpha$ -naphthylamine sulfanilic acid, modified by Lunge and Lwoff.
- ERDMANN, H., *Ber.* **33**, 210 (1900); *J. Chem. Soc.* **78**, ii, 243 (1900). Detection and estimation of very small quantities of nitrous acid. 50 cc. of the water to be tested are mixed with 5 cc. of an acidified solution of sodium sulphanilate (2 g. per liter) and about 0.5 g. 1-amino-8-hydroxynaphthalene-4 : 6 disulfonic acid added in the form of the acid sodium salt mixed with  $\text{Na}_2\text{SO}_4$ . The coloration (Bordeaux-red) reaches its maximum intensity in about an hour and may be compared colorimetrically with that produced by a known amount of a standard nitrite solution.
- ERDMANN, H., *Z. angew. Chem.* **13**, 33 (1900); *J. Soc. Chem. Ind.* **19**, 277 (1900). Detection and estimation of nitrites in potable water. Uses alkali salt of 1.1'.4.3'-amidonaphthol disulfonic acid.
- ROMIJN, G., *Chem.-Ztg.* **24**, 145 (1900); *J. Chem. Soc.* **78**, ii, 510 (1900). Estimation of nitrous acid. Criticism of Erdmann's view, etc., and his reagent. Results less satisfactory than with Griess' reagent.
- MENNICKE, H., *Z. angew. Chem.* **13**, 235 (1900); *J. Chem. Soc.* **78**, 438 (1900). Detection of nitrous acid in water by means of amino-naphthol-K-acid. Mennicke strongly recommends Erdmann's method [*Ber.* **33**, 210 (1900)].
- MENNICKE, H., *Z. angew. Chem.* **13**, 711 (1900); *J. Chem. Soc.* **78**, ii, 621 (1900); *J. Soc. Chem. Ind.* **19**, 856 (1900). Detection of nitrous acid in water. Concludes Erdmann's test most delicate of those examined and almost free from sources of error. Examined: starch-KI or Zn iodide; the 4 modifications of Griess' reagent, Riegler's reagent and Erdmann's reagent.
- SCHULTZ, G., *Chem. Zentr.* **1902**, i, 949; *Z. Farben-Textil Chem.* **1**, 37 (1902); *J. Chem. Soc.* **82**, ii, 473 (1902). Estimation of nitrous acid in sodium nitrite. Confirmation of accuracy of estimating  $\text{HNO}_2$  by means of sodium sulphanilate. Procedure not given.

- LUNGE, G., *Z. angew. Chem.* **15**, 1 (1902); *J. Soc. Chem. Ind.* **21**, 190 (1902). The supposed reaction of brucine with  $\text{HNO}_2$ . "The statement made by Winkler [*J. Chem. Soc.* **80**, ii, 627 (1901)] that brucine reacts with nitrous acid as well as with nitric acid is entirely opposed to the author's experience. He has repeated some of his experiments, which again prove that brucine only reacts with nitric acid, not with nitrous acid. If a reaction is obtained with a nitrite, this shows that the nitrous acid has been partly converted into nitric acid, a result that will always happen if the nitrous acid at the moment of its liberation does not come in contact with an excess of strong sulfuric acid so as to form the stable nitrosylsulfuric acid. If the directions given by the author and Lwoff [*J. Chem. Soc.* **66**, ii, 298 (1894)] are carefully followed, nitrous acid cannot be mistaken for nitric acid." L. de K., *J. Chem. Soc.* **82**, ii, 288 (1902).
- WINKLER, L. W., *Z. angew. Chem.* **15**, 170 (1902); *J. Chem. Soc.* **82**, ii, 353 (1902); *Analyst* **27**, 162 (1902). A reply to Lunge (*Z. angew. Chem.* **15**, 1). The author communicates a series of experiments showing that nitrites react quite as energetically as nitrates with brucine if only a moderate amount of free  $\text{H}_2\text{SO}_4$  is used.
- LUNGE, G., *Z. angew. Chem.* **15**, 241 (1902); *J. Chem. Soc.* **82**, 427 (1902). A further reply to Winkler (*Z. angew. Chem.* **15**, 170). The author now acknowledges that when using a moderate amount of  $\text{H}_2\text{SO}_4$  the brucine reaction is also given by  $\text{HNO}_2$ . He withdraws his previous remarks.
- GEELMUYDEN, H. C., *Z. anal. Chem.* **42**, 276, 518 (1903).  $\text{NO}_2$ ,  $\text{NO}_3$ , and  $\text{NH}_3$  in sea water.
- RICHARDSON, F. W. and HOLLINGS, P., *J. Soc. Chem. Ind.* **22**, 616 (1903). Use phenoldisulfonic acid for  $\text{NO}_3$  and  $\alpha$ -naphthylamine for  $\text{NO}_2$ .
- TATLOCK, R. R. and THOMSON, R. T., *J. Soc. Chem. Ind.* **23**, 429 (1904). Nitrites and nitrates in water. For  $\text{NO}_2$  use the phenolsulfonic acid method.  $\text{NO}_2$  oxidized to  $\text{NO}_3$  by  $\text{H}_2\text{O}_2$  and then determined as  $\text{NO}_3$ .
- RAIKOW, P., *Oesterr. Chem. Ztg.* **7**, 557 (1904); *Analyst* **30**, 174 (1905). The differentiation of nitric and nitrous acid by means of diphenylamine.
- WESTON, R. S., *J. Am. Chem. Soc.* **27**, 281 (1905); *J. Soc. Chem. Ind.* **24**, 350 (1905). Determination of nitrogen as nitrites in waters. Uses  $\alpha$ -naphthylamine, sulfanilic acid and  $\text{HAc}$  (instead of  $\text{HCl}$ ). Compares use of  $\text{HAc}$  and  $\text{HCl}$ .
- ALVAREZ, E. P., *Chem. News* **91**, 155 (1905). Observations on diphenylamine as reagent for nitrites, nitrates, chlorates, and its use when mixed with resorcin and  $\beta$ -naphthol. Use diphenylamine and resorcin for  $\text{NO}_2$  and  $\text{NO}_3$  and diphenylamine and  $\beta$ -naphthol for  $\text{ClO}_3$ .
- ALVAREZ, E. P., *Bull. soc. chim.* **33**, 717 (1905); *Analyst* **30**, 285 (1905). Observations on the use of diphenylamine as a reagent for nitrites, nitrates, and chlorates.
- KASTLE, J. H. and ELVOVE, E., 7th Intern. Cong. Appl. Chem., London, **1909**; *J. Soc. Chem. Ind.* **28**, 742 (1909); *C. A.* **4**, 2696 (1910). Fuchsin-S as a permanent standard for the determination of nitrites in sanitary water analysis. The pink color produced by a nitrite and Griess' sulfanilic acid-

- $\alpha$ -naphthylamine reagent can be exactly matched by a solution of acid magenta (fuchsin-S, acid fuchsin according to Weigert) which has been acidified with HCl.
- HEIM, F. and HERBERT, A., *Bull. sci. pharmacol.* **16**, 209; *Chem. Zentr.* **1909**, I, 2015. Determination of nitrogen acids in the atmosphere of workshops "Methods based on the amount of  $\text{HNO}_2$  only are not valuable. The colorimetric detn. with diphenylamine sulfate gives good results. Aspirate a definite volume, about 1 liter of the air through a 10 cc. absorption tube filled with 5% KOH, dilute the soln. to 500 cc., and mix 1 cc. in a test tube with 5 cc. of the reagent (0.2 g. diphenylamine to 1 liter of conc.  $\text{H}_2\text{SO}_4$ ) and compare the color with that produced by a std. soln. of  $\text{KNO}_3$ . One g.  $\text{KNO}_3$  corresponds to 0.455 g. or 220 cc.  $\text{HNO}_2$ ; 0.0001 cc. of  $\text{HNO}_2$  may thus be detected. Other oxidizing gases (Cl, Br, I, IIBr, HI, etc.) and organic matter must be absent." V. K. Chesnut, *C. A.* **4**, 2080 (1910).
- POUGET, I., *Bull. soc. chim.* [4], **7**, 449 (1910); *C. A.* **4**, 2424 (1910); *Z. anal. Chem.* **50**, 124 (1911). The determination of nitrites and nitrates by means of phenolsulphonic acid.
- MEYERFELD, J., *Chem.-Ztg.* **34**, 848 (1910); *C. A.* **4**, 3178 (1910). Pyrogallol dimethyl ether, a sensitive reagent for chromic acid, ferric salts and nitrous acid.
- ARMANI, G. and BARBONI, J., *Chem.-Ztg.* **34**, 994 (1910); *C. A.* **5**, 3549 (1911). A reaction for determining nitrites. Use a saturated solution of benzidine acetate and dilute acetic acid. Color ranges from yellow to red depending on the concentration.
- ROMIJN, G., *Pharm. Weekblad* **48**, 753 (1911).
- DENIGES, G., *Bull. soc. chim.* [4], **9**, 544 (1911); *J. Chem. Soc.* **100**, ii, 655 (1911); *J. Soc. Chem. Ind.* **30**, 827 (1911). Detection of nitrates and nitrites in water by means of reduced strychnine. Uses a reagent prepared by adding 5 g. of Zn amalgam to a mixture of 5 cc. HCl (sp. gr. 1.18) with 5 cc. of a 1 per cent solution of strychnine sulfate. Boil, cool, and decant. 10 cc. water containing 0.0001 g.  $\text{HNO}_2$  per liter gives a red coloration with 0.5 cc. of the reagent. Nitrates give a color only in the presence of  $\text{H}_2\text{SO}_4$ .
- DENIGES, G., *Compt. rend.* **152**, June 19 (1911); *Chem. News* **104**, 119 (1911). Rapid method of determining nitrates and nitrites in water. Uses strychnine reagent.
- TILLMANS, J. and SUTTHOFF, W., *Z. anal. Chem.* **50**, 473 (1911); *J. Soc. Chem. Ind.* **30**, 918 (1911); *C. A.* **5**, 3211 (1911). Use diphenylamine-sulfuric acid.
- VERMEHREN, A., *Centr. Zuckerind.* **19**, 72; *C. A.* **5**, 55 (1911). The determination and estimation of small quantities of nitric and nitrous acid in water. For  $\text{HNO}_2$ , the author uses Riegler's reagent, using 10 cc. of water, 10 drops of the reagent, 2 drops concentrated HCl, and then shaking with 20 drops  $\text{NH}_4\text{OH}$ . A red coloration develops. "
- WITHERS, W. A. and RAY, B. J., *J. Am. Chem. Soc.* **33**, 708 (1911); *J. Chem. Soc.* **100**, ii, 656 (1911); *J. Soc. Chem. Ind.* **30**, 708 (1911). Modification of the diphenylamine test for nitrous and nitric acid. One part of nitrous nitro-

- gen can be detected in 25 million, or 1 part of nitric nitrogen in 35 million by heating 15 to 20 min.; heating 1 hour increases the sensitiveness to 1 part in 32 million and 1 part in 44 million, respectively.
- BLANC, G., *J. pharm. chim.* **4**, 205 (1911); *J. Soc. Chem. Ind.* **30**, 1136 (1911). Examined the  $\text{ZnI}_2$  and the metaphenylenediamine- $\text{H}_2\text{SO}_4$  methods. Former rejected, the latter found satisfactory.
- MILLER, E. H., *Analyst* **37**, 345; *C. A.* **6**, 3380 (1912). Uses dimethylaniline and HCl in aqueous solution. Yellow color obtained due to *p*-nitrosodimethylaniline (15 min. to color). Can detect 1 part of  $\text{HNO}_2$  per million. Nitrates do not interfere.
- ELSDON, G. D., *Chem. News* **105**, 243 (1912); *C. A.* **6**, 2122 (1912). Determination of nitrites in potable waters. Interference of ferric salts. "In the detn. of nitrites by means of the starch-iodide reaction, the presence of ferric salts influences the results obtained. One part of ferric iron per million parts of water gives a color which corresponds with that obtained with 0.07 part of sodium nitrite; 1 part of iron in 50 million parts of water can just be detected by means of the starch-iodide reaction, while 1 part in 10 millions gives an appreciable reaction. The coloration is obtained when ferric iron and nitrates are present together. Ferrous iron does not itself yield a coloration but it diminishes the color produced by nitrites. The author prefers the Griess-Ilosvay method for the determination of nitrites." W. P. S., *J. Soc. Chem. Ind.* **31**, 600 (1912).
- PRIMOT, *Bull. sci. pharmacol.* **19**, 546 (1912). The determination of nitrous acid in water. "By comparison of color produced by EtOH soln. of benzidine, *o*-toluidine or dianisidine. Sulfates and free mineral acids must be removed." E. H. Grant, *C. A.* **7**, 1069 (1913).
- VANDEVELDE, A. J. J., Ghent. Doc. comm. intern. unif. meth. anal. denr. alim. **1912**, 118; thru *Bull. soc. chim. belg.* **26**, 422; *C. A.* **7**, 669 (1913). Rapid and approximate estimation of ammonium and nitrous ions in potable water.
- AUTENRIETH, W. and FUNK, A., *Z. anal. Chem.* **52**, 137 (1913); *C. A.* **7**, 3627 (1913). Colorimetric methods for water analysis by the use of the Autenrieth-Koenigsberger colorimeter. Details are given for the estimation of  $\text{NH}_3$ ,  $\text{HNO}_2$ ,  $\text{HNO}_3$ , Fe, Pb, and  $\text{H}_2\text{S}$ .
- BORNAND, M., *Chem. Zentr.*, **1913**, ii, 1823; from *Mett. Lebensmittelunters. Hyg.* **4**, 285 (1913); *J. Chem. Soc.* **106**, ii, 144 (1914); *C. A.* **8**, 768 (1914). Comparative investigation of certain reactions for the detection of nitrites in potable water. Compares experimentally the methods of (1) von Ilosvay-Lunge, (2) Rochaiz, (3) Barbet and Jandrier, (4) Denigès, and (5) Chwilewsky. All satisfactory except Denigès', which is unsuited for practical use on account of the instability of the reagent. Method of von Ilosvay-Lunge preferred.
- LOMBARD, M., *Bull. soc. chim.* **13**, 304 (1913); *C. A.* **7**, 2265 (1913); *J. Chem. Soc.* **104**, ii, 429 (1913); *J. Soc. Chem. Ind.* **32**, 447 (1913); *Z. anal. Chem.* **53**, 135 (1914). Practical method of determining nitrites in potable waters.
- ELSDON, G. D. and SUTCLIFFE, J. A. L., *Analyst* **38**, 450 (1913); *C. A.* **8**, 185

- (1914). Nitrates and nitrites in milk. Uses the brucine-sulfuric acid method for nitrates and the Griess-Ilosvay method for nitrites.
- LETTS, E. A. and REA, F. W., *Analyst* **39**, 350; *C. A.* **8**, 3402 (1914); *J. Soc. Chem. Ind.* **33**, 938 (1914). Fresenius' method for determining small quantities of nitrites, and its sensitiveness compared with the *m*-phenylenediamine reaction. Uses a modified Fr. method. Zn-iodide-starch. Method will detect 0.00025 mg. N in 7-8 minutes. About 20 times more sensitive than the *m*-phenylenediamine method.
- MARQUEYROL and MURAOUR, H., *Bull. soc. chim.* [IV] **15**, 186 (1914); *J. Soc. Chem. Ind.* **33**, 335 (1914). At the end of the paper they suggest that diphenylbenzidine might be substituted with advantage for diphenylamine when testing for oxidizing agents, such as nitrites, but only the suggestion is made, and no details of any such method are given.
- ROMIJN, G., *Chem. Weekblad* **11**, 115 (1914); *C. A.* **9**, 1644 (1915); *J. Soc. Chem. Ind.* **34**, 631 (1915). For the determination of  $\text{HNO}_2$  in drinking water, the ordinary colorimetric method with  $\alpha$ -naphthylamine and sulfanilic acid is modified by adding the reagent in the form of a dry powder containing  $\alpha$ -naphthylamine 1, sulfanilic acid 10 and tartaric acid 89 parts.
- LETTS, E. A. and REA, F. W., *J. Chem. Soc.* **105**, 1157 (1914); *Proc. Chem. Soc.* **30**, 72; *C. A.* **8**, 2323 (1914); *J. Soc. Chem. Ind.* **33**, 570 (1914). An extremely delicate colorimetric method for detecting and estimating nitrates and nitrites. Diphenylbenzidine used for nitrates. Nitrites oxidized by  $\text{KMnO}_4$  and similarly determined.
- ACÉL, D., *Z. Nahr. Genussm.* **31**, 332 (1916); *C. A.* **11**, 1493 (1917). Detection and quantitative determination of nitrates and nitrites in meats and sausages. The reaction between  $\text{HNO}_2$  and a solution of  $\alpha$ -naphthylamine-sulfanilic acid containing  $\text{AcOH}$  gives a red color which may be compared with standard solutions of fuchsin. Gives procedures both in presence and absence of nitrate.
- ARNY, H. V. and RING, C. H., *J. Ind. Eng. Chem.* **8**, 309 (1916); *C. A.* **10**, 1146 (1916); see also *Proc. 8th Intern. Cong. Appl. Chem.* **26**, 310; cf. Arny and Pickhardt, *Drug. Circ.* **58**, 131 (1914) and *J. Franklin Inst.* Aug. 1915. Color standards and colorimetric assays. Use colored solutions of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to prepare standard color solutions. Use sulfanilic acid and naphthylamine hydrochloride for  $\text{NO}_2$ .
- KOLTHOFF, I. M., *Utrecht. Pharm. Weekblad* **54**, 633; *C. A.* **11**, 2701 (1917). Chemical study of potable waters. III. Ammonia, nitrite and nitrate.
- OELSNER, ALICE, *Z. angew. Chem.* **31**, 170, 178 (1918); *J. Chem. Soc.* **114**, ii, 405 (1918). A survey of methods for the estimation of nitrites and nitrates in the same solution.
- QUARTAROLI, A., *Gazz. chim. ital.* **48**, I, 102 (1918); *C. A.* **13**, 1684 (1919); *Chem. Zentr.* **93**, II, 728 (1922); *Z. anal. Chem.* **62**, 469 (1923). Estimation of minute quantities of nitrites and hydrogen peroxide in presence of each other.
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**Osmium.**

TSCHUGAEFF, L., *Compt. rend.* **167**, 235 (1918); *Analyst* **43**, 362 (1918). New reaction of osmium. Uses thiourea for osmium in the state of the tetroxide,  $\text{OsO}_4$ , or a chlorosmate as  $\text{K}_2\text{OsCl}_6$ , and  $\text{HCl}$ . A rose to deep red coloration

is obtained, according to concentration. Will detect 1 part Os in 100,000 parts of solution. Color due to formation of  $[\text{Os } 6(\text{NH}_2 \cdot \text{CS} \cdot \text{NH}_2)] \text{Cl}_3\text{H}_2\text{O}$ .

#### Oxalic Acid.

SACHER, J. F., Chem.-Ztg. **39**, 319 (1915); C. A. **9**, 2043 (1915). Colorimetric detection of oxalic acid and manganese.

#### Oxybutyric Acid.

FOLIN, O. and DENIS, W., J. Biol. Chem. **18**, 263 (1914). Turbidity methods for the determination of acetone, acetoacetic acid, and  $\beta$ -oxybutyric acid in urine.

#### Oxygen.

RAMSAY, W. and HOMFRAY (Miss) I., J. Soc. Chem. Ind. **20**, 1071 (1901); J. Chem. Soc. **82**, ii, 171 (1902). Colorimetric method for determining oxygen dissolved in water. Use cuprous chloride which in ammoniacal solution is oxidized by oxygen to cupric chloride and in turn a blue copper complex is formed.

LENORMAND, C., Bull. soc. chim. **29**, 810 (1903); Analyst **28**, 307 (1903). A colorimetric method of determining the oxygen absorption of water. Method consists in boiling the water with a standard alkaline solution of  $\text{KMnO}_4$  and determining from the loss of color the amount of oxygen absorbed.

FRANKFORTER, G. B., WALKER, G. W. and Wilhoit, A. D., J. Am. Chem. Soc. **31**, 35 (1909); C. A. **3**, 1135 (1909); J. Soc. Chem. Ind. **28**, 157 (1909). Colorimetric determination of dissolved oxygen in water. Method depends upon the oxidation of cuprous-NH<sub>2</sub> chloride by exposure to oxygen. Show that am. cuprous chloride method of Ramsay and Homfray gives accurate results if precautions are taken to prevent oxidation of the cuprous chloride.

RIDEAL, S. and BURGESS, W. T., Analyst **34**, 193 (1909). Estimation of oxygen dissolved in water.

WINKLER, L. W., Z. angew. Chem. **24**, 341 (1911); J. Chem. Soc. **100**, ii, 329 (1911). Estimation of dissolved oxygen. Method based upon a brownish-yellow color produced when water containing dissolved oxygen is treated with ammonia and a derivative of quinol (a photographic developer known as "adurol").

WINKLER, L. W., Z. angew. Chem. **24**, 831 (1911); J. Chem. Soc. **100**, ii, 532 (1911). Estimation of dissolved oxygen. Suggests using as a standard a 2-N NaCl solution saturated with air. Solubility data are given.

WINKLER, L. W., Z. angew. Chem. **26**, I, 134 (1913). Borax, adurol, and Rochelle salt give a reagent that may be used in place of adurol. The Rochelle salt prevents precipitation of Ca.

SNELL, F. D., Colorimetric Analysis, p. 129, D. Van Nostrand Co., New York, 1921. Determination of oxygen by cuprous chloride.

SNELL, F. D., *ibid.*, p. 132, 1921. Determination of oxygen by adurol.

SHEAFF, H. M., J. Biol. Chem. **52**, 35 (1922). Estimation of minute amounts of gaseous oxygen and its application to respiratory air. The oxygen is converted into  $\text{NaNO}_2$  in the presence of nitric oxide and NaOH and the nitrite then estimated by means of sulfanilic acid and  $\alpha$ -naphthylamine.



HAND, P. G. T., J. Chem. Soc. **123**, 2573 (1923); C. A. **18**, 207 (1924); J. Soc. Chem. Ind. **42**, 1221A (1923); Z. anal. Chem. **64**, 353 (1924). Uses a modification of Rideal's and Burgess' [Analyst **34**, 193 (1909)] colorimetric method which consists in bringing  $O_2$  in contact with  $Mn(OH)_2$  formed by  $MnCl_2$  and NaOH in a vacuum in presence of KI. Acidify in presence of starch and match color. Limit of application is  $1 \times 10^{-7}$  g.  $O_2$ . Diagram of apparatus is given.

EFIMOV, V. V., Biochem. Z. **155**, 371 (1925). Colorimetric method for the determination of oxygen. "Indigo carmine is dissolved in  $H_2O$  to give a 0.1% soln. Then glucose and  $K_2CO_3$  are added to give a 1% soln. of each. In 24 hrs., the leuco base is formed, as shown by decolorization of the soln. Vaseline oil is poured in to form a layer over the  $H_2O$  and the flask is sealed. Special capillary pipettes are used to remove the leuco base. By using varying amts. of leuco base in the same vols. of  $H_2O$ , and exposing them to air different color standards are obtained. The soln. to be tested for O is placed with min. agitation in a tube, covered with oil, and a known amt. of leuco base added. The color developed is compared with the standards against a  $K_2Cr_2O_7$  soln. as a background. To convert to mg. O, a comparison with the method of Winkler is made. The method has the advantage of being short." W. D. Langley, C. A. **19**, 3504 (1925).

#### Pentosans.

TESTONI, G., Staz. sper. agrar. ital. **50**, 97 (1917); from Chem. Zentr. **1918**, ii, 865; J. Chem. Soc. **116**, ii, 122 (1919). Quantitative colorimetric estimation of pentosans in meal. Uses HAc and HCl, and 0.25 per cent phloroglucinol. Red solution obtained.

#### Pepsin.

GRÜTZNER, P., Pflüger's Arch. ges. Physiol. **8**, 452 (1874); J. Chem. Soc. **27**, 609 (1874).

GRÜTZNER, P. v., Arch. Fisiol. Fanós Festschr. **7**, 223; Z. entr. Biochem. Biophys. **12**, 379; C. A. **6**, 2630 (1912).

SMORODINZEV, I. A. and ADOVA, A. N., Hoppe-Seyler's Z. Physiol. Chem. **149**, 173 (1925); C. A. **20**, 214 (1926). Preparation of standards for the colorimetric determination of pepsin.

#### Perchlorate.

MONNIER, A., Arch. Sci. Phys. Nat., Genève [4] **42**, 210 (1916); Chem. Zentr. **88**, ii, 132 (1917). Determination of perchlorates by methylene blue.

SNELL, F. D., Colorimetric Analysis, p. 106, D. Van Nostrand Co., New York, 1921. Determination of perchlorate by methylene blue.

#### Phenol.

CARRÉ, L., Compt. rend. **113**, 139 (1891).

BACH, H., Z. anal. Chem. **50**, 736 (1911); C. A. **6**, 586 (1912); J. Soc. Chem. Ind. **30**, 1368 (1911). Colorimetric determination of phenols in waste

- waters. Method depends upon color given by Millon's reagent and few drops  $\text{HNO}_3$ , with phenol. Best results with phenol 30 to 150 mg. per liter.
- FOLIN, O. and DENIS, W., *J. Biol. Chem.* **12**, 239 (1912). On phosphotungstic-phosphomolybdic compounds as color reagents.
- MUMFORD, E. M., *Chem. News* **107**, 253 (1913). The estimation of phenol in presence of organic matter. Produces phenol sulphonic acid, nitrates the latter and makes alkaline with ammonia, thus forming  $\text{NH}_4$  picrate. The yellow color is then matched against a standard solution.
- LEWIS, H. B. and NICOLET, B. H., *J. Biol. Chem.* **16**, 369 (1913-14).
- MILLER, C. W. and TAYLOR, A. E., *J. Biol. Chem.* **17**, 531 (1914). Suggest that the reagent (30 g. ammonium molybdate and 25 cc.  $\text{H}_2\text{SO}_4$  dissolved in 1 liter of distilled water) might be developed to serve for the colorimetric estimation of the indole and phenol derivatives contained in urine.
- FOLIN, O. and DENIS, W., *J. Biol. Chem.* **22**, 305 (1915); *J. Soc. Chem. Ind.* **34**, 1111 (1915). A colorimetric method for the determination of phenols (and phenol derivatives) in urine.
- DUBIN, H., *J. Biol. Chem.* **26**, 69 (1916). Physiology of the phenols.
- FOLIN, O. and DENIS, W., *J. Biol. Chem.* **26**, 507 (1916). The relative excretion of phenols by the kidneys and by the intestine. Total phenols with phosphotungstic-phosphomolybdic acid reagent.
- GREENWALD, I. and WEISS, M. L., *J. Biol. Chem.* **31**, 1 (1917). The fate of inositol administered to dogs. Phenols by Folin and Denis method [*J. Biol. Chem.* **22**, 305 (1915)].
- ELVOVE, E., U. S. Pub. Health Serv. Hyg. Lab. Bull. **110**, 25 (1917); *C. A.* **12**, 708 (1918). A colorimetric method for the estimation of the cresol or phenol preservative in serums.
- BENEDICT, S. R. and THEIS, R. C., *J. Biol. Chem.* **36**, 95 (1918). The colorimetric determination of phenols in the blood.
- WEISS, M., *Biochem. Z.* **110**, 258 (1920); *J. Chem. Soc.* **118**, ii, 777 (1920); *C. A.* **15**, 1913 (1921). The colorimetric estimation of phenol in urine. Uses Millon's reagent.
- CHAPIN, R. M., *J. Ind. Eng. Chem.* **12**, 771 (1920); *J. Chem. Soc.* **118**, ii, 645 (1920); *C. A.* **14**, 2772 (1920); *J. Soc. Chem. Ind.* **39**, 652A (1920). Estimation of phenol in the presence of certain other phenols. Uses Millon's reagent. Various modifications of the method are described which are suitable for different requirements.
- UNDERHILL, F. P. and SIMPSON, G. E., *J. Biol. Chem.* **44**, 69 (1920). The effect of diet on the excretion of indican and the phenols. Phenols by Folin-Denis method.
- TISDALL, F. F., *J. Biol. Chem.* **44**, 409 (1920). Estimation of the phenolic substances in urine. Folin and Denis method may give results 50 per cent higher than those obtained with the ether extraction method outlined in this paper.
- SCOTT, R. D., *J. Ind. Eng. Chem.* **13**, 422 (1921). The detection of phenols in water. Uses phosphomolybdic acid.
- PELKAN, K. F., *J. Biol. Chem.* **50**, 491 (1922). The physiology of the phenols.

- A quantitative method for the determination of phenols in the blood. Uses sodium tungstate.
- THOMAS, P., *Ann. inst. Pasteur* **36**, 253 (1922); *Ber. ges. Physiol. exptl. Pharmacol.* **13**, 376 (1922). Estimation of tyrosin and phenol index of proteins.
- HENNINGSSEN, C., *Ind. Eng. Chem.* **15**, 406 (1923). Estimation of various monohydric phenols by the phenol reagent of Folin and Denis.
- RAKESTRAW, N. W., *J. Biol. Chem.* **56**, 109 (1923). A quantitative method for the determination of phenols in blood. Uses a modified Folin and Denis method.
- GOIFFON, R. and NEPVEUX, F., *Compt. rend. soc. biol.* **89**, 1213 (1923); from *Chem. Zentr.* **1924**, i, 1070; *J. Chem. Soc.* **126**, ii, 706 (1924); *C. A.* **18**, 2015 (1924). Colorimetric determination of phenols in urine. Use method of Rakestraw.
- THEIS, R. C. and BENEDICT, S. R., *J. Biol. Chem.* **61**, 679 (1924). Determination of phenols in blood. Add *p*-nitrobenzenediazonium chloride to the protein-free filtrate, by the Folin and Wu method, containing  $\text{Na}_2\text{CO}_3$ . Orange color produced. Possible error  $\pm 10$  per cent.
- CRISTOL, P., *Bull. soc. sci. m d. Montpellier* **9**, 456 (1924); *Physiol. Abstracts* **9**, 465; *C. A.* **19** (1925). Errors in colorimetric methods of estimation of uric acid and phenols in urine. See the abstract of this paper under **Uric Acid**.
- VORCE, L. R., *Ind. Eng. Chem.* **17**, 751 (1925); *C. A.* **20**, 83 (1926). Determination of minute amounts of phenols in polluted natural waters. Uses the Folin and Denis reagent.
- HAWK, P. B. and BERGEIM, O., *Practical Physiological Chemistry*, 9 ed., P. Blakiston's Son and Co., Philadelphia, **1926**. Determination of phenols in urine, pp. 766-768.

#### **Phenolsulfonephthalein.**

- UNDERHILL, F. P. and BLATHERWICK, N. R., *J. Biol. Chem.* **19**, 39 (1914). The elimination of phenolsulfonephthalein in acute and chronic tartrate nephritis. Use an Autenrieth-Koenigsberger colorimeter to determine the amount of coloring matter excreted.

#### **Phosphate (and Phosphoric Acid).**

- KNIGHTS, J. W., *Analyst* **5**, 197 (1880); *Z. anal. Chem.* **21**, 572 (1882). First to propose use of  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ .
- OSMOND, F., *Bull. soc. chim.* **47**, 745 (1887); *J. Chem. Soc.* **52**, 999 (1887); *J. Anal. Chem.* **1**, 421 (1887). Method is based upon the blue color formed by P molybdates in HCl solution of  $\text{SnCl}_2$ . As and Si molybdates give the same color.
- B NAZET, P., *Bull. soc. chim.* **11**, 1083 (1894); *J. Soc. Chem. Ind.* **14**, 390 (1895). Estimation of phosphorus in iron and steel, etc. Dissolves the phosphomolybdate in  $\text{SnCl}_2$  in dilute HCl solution.
- ALESSANDRIA, *Pharm. Centralhalle* **35**, 109; *Chem. News* **74**, 45 (1896). Colorimetric determination of phosphoric acid in water. First removes  $\text{SiO}_2$  and then adds "molybdenic mixture."

- LEPIERRE, C., *Bull. soc. chim.* **15**, 1213 (1896); *J. Soc. Chem. Ind.* **16**, 163 (1897). Removes  $\text{SiO}_2$  by dehydration and then adds  $\text{NH}_4$  molybdate.
- JOLLES, A., *Chem.-Ztg.* **1889**, 807.
- JOLLES, A. and NEURATH, F., *Monatsh.* **19**, 5 (1898); *J. Soc. Chem. Ind.* **17**, 493 (1898). Remove  $\text{SiO}_2$  by dehydration and then add K molybdate.
- JOLLES, A., *Prager med. Wochschr.* **1899**, 307; *Wiener med. Wochschr.* **1899**.
- JOLLES, A. and NEURATH, F., *Arch. Hyg.* **34**, 22 (1899); *J. Soc. Chem. Ind.* **18**, 299 (1899). Removes  $\text{SiO}_2$  by dehydration and then adds K molybdate.
- JOLLES, A., *Chem. Zentr.* **1899**, i, 375; *J. Chem. Soc.* **76**, ii, 579 (1899). Colorimetric estimation of phosphoric acid in water. Uses K molybdate in  $\text{HNO}_3$ . Turbid solution is obtained at  $30^\circ$  if more than 1 mg.  $\text{P}_2\text{O}_5$  is present in 20 cc.
- WOODMAN, A. G. and CYVAN, L. L., *J. Am. Chem. Soc.* **23**, 96 (1901); *J. Soc. Chem. Ind.* **20**, 506 (1901). The determination of phosphates in potable waters. Use am. molybdate and  $\text{HNO}_3$  and study the effect of  $\text{SiO}_2$ .
- WOODMAN, A. G., *J. Am. Chem. Soc.* **24**, 735 (1902). The significance of phosphates in natural waters. Uses  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ , after dehydrating the  $\text{SiO}_2$  by heating the residue for 2 hrs. at  $100^\circ$ . Not necessary to filter.
- VEITCH, F. P., *J. Am. Chem. Soc.* **25**, 169 (1903). On the colorimetric determination of small quantities of phosphoric acid and of silica. Uses  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ . Both  $\text{PO}_4$  and  $\text{SiO}_2$  give a yellow coloration but there is a difference in the rate of reaching the maximum intensity of color.
- SCHREINER, O., *J. Am. Chem. Soc.* **25**, 1056 (1903). On a colorimetric method for the estimation of phosphates in the presence of silica. Uses  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ .
- SCHREINER, O., *J. Am. Chem. Soc.* **26**, 808 (1904). The determination of phosphates in aqueous extracts of soils and plants. Reports results by method given in *J. Am. Chem. Soc.* **25**, 1056 (1903) and also results of gravimetric method of Asscn. Official Agr. Chem. A comparison of the two methods. Agreement good.
- LINCOLN, A. T. and BARKER, P., *J. Am. Chem. Soc.* **26**, 975 (1904). Determination of phosphates in natural waters. Use  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ .
- SCHREINER, O. and BROWN, B. E., *J. Am. Chem. Soc.* **26**, 1463 (1904); *J. Soc. Chem. Ind.* **23**, 1240 (1904). The colorimetric estimation of phosphates; second method. Use  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ . Method gives good results with 1 part  $\text{P}_2\text{O}_5$  per million of solution and is not influenced by the presence of other salts.
- HEWITT, T. E., *Proc. Eng. Soc. West. Penn.* **20**, 432 (1904); *Analyst* **30**, 137 (1905); see also *J. Am. Chem. Soc.* **27**, 121 (1905). Colorimetric determination of phosphorus in iron and steel. Based on the color obtained when  $\text{H}_2\text{S}$  is passed into an alkaline phosphomolybdate solution.
- HEWITT, T. E., *J. Am. Chem. Soc.* **27**, 121 (1905); *J. Soc. Chem. Ind.* **24**, 292 (1905). Colorimetric determination of phosphorus in iron.  $\text{H}_2\text{S}$  passed into alkaline solution of Na phosphomolybdate and the resulting color matched against a standard solution.
- MISSON, G., *Chem.-Ztg.* **32**, 633 (1908); *C. A.* **2**, 2527 (1908). Uses  $\text{KMnO}_4$ ,  $\text{H}_2\text{O}_2$ ,  $(\text{NH}_4)_3\text{VO}_4$ , and  $(\text{NH}_4)_2\text{MoO}_4$ . P estimated to about 0.005 per cent.

- POUGET, I., *Chem.-Ztg.* **32**, 832 (1908); *Analyst* **33**, 441 (1908). Rapid colorimetric method for the estimation of small quantities of water-soluble phosphoric acid. Uses strychnine molybdate. Method sensitive to 1 part  $\text{H}_3\text{PO}_4$  in 12,000,000 of water.
- POUGET, I. and CHOUGHAK, D., *Ann. chim. anal. chim. appl.* **14**, 125; *C. A.* **3**, 2284 (1909). Uses  $\text{Na}_2\text{MoO}_4$  in  $\text{HNO}_3$  and strychnine sulfate.
- ESTES, C., *J. Am. Chem. Soc.* **31**, 247 (1909); *J. Chem. Soc.* **96**, ii, 266 (1909); *J. Soc. Chem. Ind.* **28**, 261 (1909). Colorimetric estimation of phosphates in solution with other salts. Uses phosphomolybdate method and studies effect of the presence of certain salts on the color.
- GIBSON, R. B. and ESTES, C., *J. Biol. Chem.* **6**, 349 (1909); *J. Soc. Chem. Ind.* **28**, 962 (1909); *Z. anal. Chem.* **51**, 67 (1912). The indirect colorimetric determination of phosphorus with uranium acetate and potassium ferrocyanide. P precipitated as uranium phosphate and the excess of uranium acetate determined colorimetrically by adding K ferrocyanide. Red-brown color in about 2 minutes.
- POUGET, I., *Bull. soc. chim.* [4] **5**, 104 (1909); *C. A.* **3**, 1258 (1909); *J. Soc. Chem. Ind.* **28**, 261 (1909). Uses  $\text{Na}_2\text{MoO}_4$  in  $\text{HNO}_3$  and strychnine sulfate solution. Method depends upon the fact that phosphomolybdic acid forms an insoluble precipitate with alkaloids. Sensitive to 0.005 mg. in 100 cc. Reaction not influenced by  $\text{SiO}_2$  and the various oxides. P can be determined in iron ores containing 1200 times as much Fe as  $\text{P}_2\text{O}_5$ .
- POUGET, I. and CHOUGHAK, D., *Bull. soc. chim.* **9**, 649 (1911); *C. A.* **5**, 3208 (1911); cf. *C. A.* **3**, 1258, 2284; *J. Soc. Chem. Ind.* **30**, 985 (1911). Method based on insolubility of strychnine phosphomolybdate. Application to very small amounts of  $\text{P}_2\text{O}_5$  in natural waters, animal and vegetable substances, minerals, iron and steel.
- PASSERINI, N., *Gazz. chim. ital.* **41**, I, 182; *C. A.* **5**, 2377 (1911). Molybdate-gallic acid method applied to substances containing very small amounts  $\text{H}_3\text{PO}_4$  (soil and rocks). P precipitated as  $\text{NH}_4$  phosphomolybdate, first dissolved in  $\text{NH}_4\text{OH}$  (1 : 3),  $\text{NH}_3$  boiled off, and solution run into tube containing 2 cc. cold, saturated gallic acid until yellow color matches that of a standard.
- HERRERA, C. R., *Anales. inst. med. nacional* **10**, 271; *C. A.* **6**, 662 (1912). Colorimetric determination of phosphoric acid in arable soils.
- RIEGLER, E., *Bull. acad. sci. Roumanie* **2**, 272 (1914); *J. Chem. Soc.* **108**, ii, 368 (1915); *C. A.* **9**, 3043 (1915); *Chem. Zentr.* **1915**, I, 1280; *J. Soc. Chem. Ind.* **34**, 796 (1915). P precipitated as  $\text{NH}_4$  phosphomolybdate and reduced with hydrazine sulfate. Blue color obtained.
- TAYLOR, A. E. and MILLER, C. W., *J. Biol. Chem.* **18**, 215 (1914). On the estimation of phosphorus in biological material. Use phenylhydrazine for determining minute traces of phosphoric acid by means of the colorimeter.
- MEDINGER, P., *Chem.-Ztg.* **33**, 781 (1915); *C. A.* **10**, 27 (1916). Detection and estimation of traces of phosphoric acid, especially in potable water. Uses a reagent of  $\text{NH}_4$  molybdate and strychnine nitrate in strong  $\text{HNO}_3$ .

- GREENWALD, I., J. Biol. Chem. **21**, 29 (1915). The estimation of lipid and acid-soluble phosphorus in small amounts of serum.
- MILLAR, C. E. and GANGLER, F. A., J. Ind. Eng. Chem. **7**, 619 (1915); J. Chem. Soc. **108**, ii, 649 (1915). Colorimetric estimation of phosphorus in soil extracts. Use Veitch's method as modified by Schreiner and Brown. Results satisfactory.
- GREENWALD, I., J. Biol. Chem. **25**, 431 (1916). The nature of the acid-soluble phosphorus of serum. Total "acid soluble" phosphorus determined colorimetrically.
- ARNY, H. V. and RING, C. H., J. Ind. Eng. Chem. **8**, 309 (1916); C. A. **10**, 1146 (1916); see also Proc. 8th Intern. Cong. Appl. Chem. **26**, 319; cf. Arny and Pickhardt, Drug. Circ. **58**, 131 (1914) and J. Franklin Inst. Aug. 1915. Color standards and colorimetric assays. Use colored solutions of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to prepare standard color solutions. Use  $\text{NH}_4$  molybdate reagent for P.
- HOWLAND, J., HAESSLER, F. H. and MARRIOTT, W. McK., Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **24**, xviii (1916). The use of a new reagent for microcolorimetric analysis as applied to the determination of calcium and of inorganic phosphates in the blood serum. Use  $\text{Fe}(\text{CNS})_3$  method. See abstract of this reference under **Calcium**.
- MARRIOTT, W. McK. and HAESSLER, F. H., J. Biol. Chem. **32**, 241 (1917). A micro method for the determination of inorganic phosphates in the blood serum. Use ferric thiocyanate.
- VAN ECK, P. N., Pharm. Weekblad **55**, 1037 (1918); J. Chem. Soc. **114**, ii, 370 (1918). Colorimetric estimation of phosphoric acid in potable water. Uses  $\text{SnCl}_2$ . Blue coloration obtained.
- SATO, A., J. Biol. Chem. **35**, 473 (1918). A rapid micro method for the determination of phosphate and total phosphorus in urine and stools. Uranium phosphate.
- BLOOR, W. R., J. Biol. Chem. **36**, 33 (1918). Methods for the determination of phosphoric acid in small amounts of blood. Uses strychnine molybdate reagent.
- FEIGL, J., Biochem. Z. **92**, 39 (1918).
- SCHRÖDER, R., Stahl u. Eisen **38**, 316 (1918); C. A. **14**, 1795 (1920). Colorimetric determination of phosphorus in steel. A yellow solution of phosphovanadio-molybdate is formed.
- KLEINMANN, H., Kolloid-Z. **27**, 236 (1920). Über die Bestimmung der Phosphorsäure, Inaugural-Dissertation (Berlin, 1919).
- KLEINMANN, H., Biochem. Z. **99**, 37 (1919). Uranium phosphate method.
- KLEINMANN, H., Biochem. Z. **99**, 42 (1919). Studies the influence of  $\text{NH}_4\text{Cl}$ ,  $\text{NaCl}$ ,  $\text{HCl}$ , and  $\text{H}_2\text{SO}_4$  on the colorimetry of P, Mo, and V compounds.
- KLEINMANN, H., Biochem. Z. **99**, 60, 61 (1919).
- KLEINMANN, H., Biochem. Z. **99**, 115, 150 (1919).
- BELL, R. D. and DOISY, E. A., J. Biol. Chem. **44**, 55 (1920); C. A. **14**, 3685 (1920). Rapid colorimetric methods for the determination of phosphorus

- in urine and blood. Use  $(\text{NH}_4)_2\text{MoO}_4$ , hydroquinone, and  $\text{Na}_2\text{CO}_3\text{-Na}_2\text{SO}_4$  solution.
- DÉBOURDEAUX, L., Bull. sci. pharmacol. **27**, 70 (1920); J. Chem. Soc. **118**, ii, 505 (1920); C. A. **14**, 1796 (1920). Modifies Denigès' strychnine-molybdate reagent in order to detect phosphate in the presence of barium.
- GRÉGOIRE, A., Bull. soc. chim. Belg. **29**, 253 (1920); C. A. **16**, 2093 (1922). Uses molybdic acid-quinine reagent.
- BLOOR, W. R., with the assistance of E. D. FARRINGTON, J. Biol. Chem. **45**, 171 (1920-21). Blood phosphates in the lipemia produced by acute experimental anemia in rabbits. Modifications of methods in J. Biol. Chem. **36**, 33 (1918).
- JONES, M. R. and NYE, L. L., J. Biol. Chem. **47**, 321 (1921). The distribution of calcium and phosphoric acid in the blood of normal children. Phosphoric acid by Bloor's method with few minor changes.
- FISKE, C. H., J. Biol. Chem. **49**, 171 (1921). Inorganic phosphate and acid excretion in the post absorptive method. Inorganic phosphate by Bell-Doisy method.
- LIZINS, J. L., Pharm. J. **106**, 478 (1921); J. Chem. Soc. **120**, ii, 518 (1921). Estimation of small quantities of phosphates in glycerophosphates. Uses  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ .
- FLORENTIN, D., Ann. chim. anal. **3**, 295 (1921); J. Chem. Soc. **120**, ii, 707 (1921); Analyst **46**, 511 (1921). Estimation of phosphates in waters. Uses  $\text{NH}_4$  molybdate in  $\text{H}_2\text{SO}_4$ , and  $\text{SnCl}_2$ . Blue color obtained.
- SNELL, F. D., Colorimetric Analysis, p. 121, D. Van Nostrand Co., New York, 1921. Determination of phosphorus as the phosphomolybdate.
- SNELL, F. D., *ibid.*, p. 122, 1921. Determination of phosphorus, separated by precipitation as  $\text{MgNH}_4\text{PO}_4$ .
- MYERS, B. A. and SHEVKY, M. C., J. Lab. Clin. Med. **7**, 176 (1921-22). The estimation of inorganic phosphorus in blood plasma by the method of Bell and Doisy.
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- DENIS, W. and MEYSENBURG, L. VON, J. Biol. Chem. **52**, 1 (1922). Note on a possible source of error in the Bell-Doisy method for the determination of phosphates in blood plasma.
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- on the blue reduction product formed when  $(\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_3$  is treated with hot  $\text{Na}_2\text{S}_2\text{O}_3$  solution.
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- HAMILTON, B., J. Biol. Chem. **65**, 101 (1925). Inorganic phosphates by Briggs' method.
- HOLT, L. E., Jr., with the assistance of I. GITTLEMAN, J. Biol. Chem. **66**, 23 (1925). The solubility of tertiary calcium phosphate in cerebrospinal fluid. P by Briggs-Bell-Doisy method.
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- LOHMANN, K. and JENDRASSIK, L., Biochem. Z. **178**, 419 (1926); C. A. **21**, 1286 (1927). Colorimetric determination of phosphoric acid in muscle extract.

ATKINS, W. R. G. and WILSON, E. G., *Biochem. J.* **20**, 1223 (1926); *C. A.* **21**, 1778 (1927). Colorimetric estimation of minute amounts of compounds of silicon, of phosphorus and of arsenic.

#### Picric Acid.

CHRISTEL, G., *Chem. News* **50**, 60 (1884); *Am. J. Pharm.* **58**, 212 (1884); *Arch. der Pharm.* [3] **21**, 190. Detection and determination of picric acid. Uses KCN to form phenylpurpuric acid from the substance under examination and matches the color against a standard picric acid solution similarly treated with KCN.

RICHARDSON, F. W., *J. Soc. Chem. Ind.* **33**, 13 (1917); *C. A.* **11**, 1383 (1917). Estimation of free sulfuric, nitric and picric acids in presence of each other. Uses the Lovibond tintometer for the picric acid,  $\text{HNO}_3$  estimated by the phenoldisulfonic acid method, and total acidity by using Me-red.

LAPORT, X., *Bull. soc. pharm. Bordeaux*, No. 3, 1917; from *Ann. chim. anal.* **23**, 64 (1918); *J. Chem. Soc.* **114**, ii, 179 (1918). Colorimetric estimation of picric acid and its derivatives in body fluids. Uses ferrous sulfate-tartaric acid reagent and ammonia.

#### Pigments, Bile.

SABATINI, G., *Klin. Wochschr.* **2**, 2031 (1923); *Chem. Zentr.* **1924**, i, 1425. Determination of bile pigments in urine.

#### Pigments, Blood.

STADIE, W. C., *J. Biol. Chem.* **41**, 237 (1920).

#### Pinolin.

GRIMALDI, C., *Chem.-Ztg.* **31**, 1145; *C. A.* **2**, 977 (1908). A color reaction is described which permits the detection of 5 per cent of pinolin or rosin spirit mixed with turpentine oil or 10 per cent mixed with pine tar oil. A second color reaction described is a modification of the Halphen test for rosin in wood spirit (*J. pharm. chim.* **1902**, 408). The reagent consists of 3 cc. bromine dissolved in  $\text{CCl}_4$  to make 15 cc. With this reagent it is possible to detect less than 1 per cent of pinolin.

#### Pitch.

LEO, K., *Chem.-Ztg.* **33**, 359 (1909); *Analyst* **34**, 286 (1909). The colorimetric estimation of pitch in fuel briquettes. A rapid colorimetric method based upon the fact that benzene dissolves the bitumen in pitch, forming a brown solution, the intensity of the color of which is proportional to the amount of bitumen.

#### Platinum.

MINGAYE, J. C. H., *Records Geol. Survey N. S. Wales* **8**, 276 (1909). Uses  $\text{SnCl}_2$  or KI. As little as 0.06 g. Pt per ton may be estimated.

ARDAGH, E.-G. R., SEABORNE, F. S. and GRANT, N. S., *Can. Chem. Met.* **8**, 117, 140 (1924); *C. A.* **18**, 2664 (1924). The colorimetric determination of platinum by potassium iodide. Red color due to  $\text{PtI}_4^{--}$  ions.

**Potassium.**

- MORRELL, T. T., J. Am. Chem. Soc. **2**, 145 (1880). Suggests the use of  $\text{PtCl}_4$  to estimate minute quantities of potassium based upon the following reaction:  $6\text{KI} + \text{PtCl}_4 \rightarrow \text{K}_2\text{PtI}_6 + 4\text{KCl}$ . No experimental data given. A red coloration obtained.
- MYLIUS, F. and FOERSTER, F., Z. anal. Chem. **31**, 250 (1892).
- HILL, L. A., J. Am. Chem. Soc. **25**, 990 (1903); J. Soc. Chem. Ind. **22**, 1152 (1903). K precipitated by chlorplatinic acid as K chlorplatinate and the latter reduced by  $\text{SnCl}_2$  in presence of free  $\text{HCl}$ . Sensitive to 1 part of  $\text{K}_2\text{O}$  per million of solution.
- CAMERON, F. K. and FAILYER, G. H., J. Am. Chem. Soc. **25**, 1063 (1903). The determination of small amounts of potassium in aqueous solutions. K precipitated as K platonic chloride and excess of KI added to the washed precipitate. A pink to rose color develops.
- ORLOW, N. A., Farmaz. J. **42**, 1737 (1903); Chem.-Ztg. Rep. **28**, 36; Analyst **29**, 201 (1904). On the quantitative determination of potassium in mineral waters. A modification of Hill's method, J. Am. Chem. Soc. **25**, 990 (1903). Acidifies  $\text{K}_2\text{PtCl}_6$  solution with  $\text{HCl}$  and adds KI.
- SNELL, F. D., Colorimetric Analysis, p. 83, D. Van Nostrand Co., New York, 1921. Potassium by determination of the potassium platino chloride by reduction with  $\text{SnCl}_2$ .
- SNELL, F. D., *ibid.*, p. 85, 1921. Potassium as the chlorplatinate by KI.
- YOSHIMATSU, S., Tōhoku J. Exptl. Med. **8**, 174 (1926). Determination of potassium with 0.2 cc. of blood. "K is pptd. as K cobaltinitrite either in the  $\text{AcOH}$  soln. of the blood ash or in undild. serum, by the addn. of an excess of Na cobaltinitrite. The ppt. is dissolved in  $\text{HNO}_3$  and then reduced by the addn. of dimethylglyoxime and  $\text{Na}_2\text{S}$ . The color thus developed is applied colorimetrically. The results on 0.2 cc. of blood or 1 cc. of serum are within 5 per cent." L. W. R., C. A. **21**, 1133 (1927).

**Protein.** (See also **Albumin.**)

- LINTNER, C. J., Z. ges. Brauw. **30**, 293; C. A. **1**, 2741 (1907); J. Soc. Chem. Ind. **26**, 705 (1907). Colorimetric estimation of the protein of barley by Millon's reagent.
- KANTOR, J. L. and GIES, W. J., Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **9**, xvii (1909). Additional experiments with the Biuret reagent. Colorimetric quantitative determinations of protein.
- AUTENRIETH, W. and MINK, F., Münch. med. Wochschr. **62**, 1417 (1915); Chem. Zentr. 1265 (1915). Urinary proteins.
- FRANKEL, E. M., J. Biol. Chem. **26**, 31 (1916). A comparative study of the behavior of purified proteins towards proteolytic enzymes.
- BLOOM, M. and POMARET, M., Compt. rend. soc. biol. **84**, 354 (1921); Ber. ges. Physiol. exptl. Pharmakol. **7**, 211 (1921). A turbidimetric standard for protein determination of spinal fluids.
- WU, H., J. Biol. Chem. **51**, 33 (1922); C. A. **16**, 2157 (1922). A new colori-

metric method for the determination of plasma proteins. Uses phosphomolybdic acid.

SWANSON, W. W., *J. Biol. Chem.* **62**, 565 (1924-25). Plasma proteins by Wu's method.

HAWK, P. B. and BERGEIM, O., *Practical Physiological Chemistry*, 9 ed., P. Blakiston's Son and Co., Philadelphia, 1926. Determination of blood proteins, pp. 398-400; proteins in urine, pp. 756-757.

#### Prussian Blue.

KNIGHT, G. W., *J. Ind. Eng. Chem.* **6**, 909 (1914).

#### Quinine.

ROY, A. C., *Indian J. Med. Research* **14**, 129 (1926). The estimation of minute quantities of quinine in the blood. "Tanret's reagent does not give good results in concns. higher than 0.025 mg. in 5 cc. Wagner's reagent gives too deep a yellow for delicate comparisons. 'An acidified 0.01 N I soln. gives very good results in detecting a difference of 0.002 mg. in the alkaloid contents between the ranges of 0.045 mg. in 5 cc. to 0.03 mg. in 5 cc. and a difference of 0.001 mg. between 0.03 and 0.001. With 5 cc. as total volume, even the presence of 0.0005 mg. can be detected when matched against 5 cc. of a satd.  $(\text{NH}_4)_2\text{SO}_4$  soln. With 2 cc. as total vol., the presence of 0.0001 mg. can be detected.'" F. Krasnow, *C. A.* **21**, 111 (1927).

#### Rhodium.

IVANOV, V. N., *J. Russ. Phys.-Chem. Soc.* **49**, 601; **50**, 460 (1917-18); *C. A.* **18**, 1448 (1924); *Z. anal. Chem.* **64**, 408 (1924). A new reaction for rhodium and a colorimetric method for its estimation. Aqueous solutions of Rh salts give, when mixed with  $\text{SnCl}_2$ , heated to boiling, and cooled, brown, colloidal solutions of the metal (similar to those of Au and Pt) which develop a fine crimson color on standing. Compare tint with standards similarly prepared. Can detect 0.0005 g. Rh per liter.

#### Saccharine.

BLOOR, W. R., *J. Biol. Chem.* **8**, 227 (1910-11). A method for determination of saccharine in urine. Uses phenol-sulfonic acid. Saccharine probably transformed into phenol-sulfonaphthalein or sulfurein. A yellow colored solution is obtained.

WAKEMAN, A. J., *J. Biol. Chem.* **8**, 233 (1910-11). Estimation of saccharine in urine and feces. Uses ethyl acetate as a solvent instead of benzol, and lead acetate in the place of sodium acetate. (Cf. Bloor, *J. Biol. Chem.* **8**, 227 (1910-11)).

#### Saffron.

VINASSA, E., *Arch. Pharm.* **230**, 353 (1892).

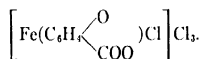
#### Salicylic Acid.

MUTER, *Analyst* **1**, 193 (1876). Uses  $\text{FeCl}_3$ .

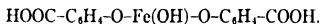
RÉMONT, A., *J. phar. chim.* July, 1881; *Chem. News* **44**, 226 (1881). Determination of salicylic in beverages.

- PELLET, H. and GROBERT, J. DE, Chem. News **44**, 168 (1881); Compt. rend. No. 5, Aug. 1, 1881. Colorimetric determination of salicylic acid in alimentary substances. Use  $\text{FeCl}_3$ .
- PELLET, H. and GROBERT, M. DE, Rev. des Ind. et des Sci. Chim. et Agri. No. 53, **1882**; Chem. News **45**, 264 (1882). Determination of salicylic acid in alimentary substances. Use  $\text{FeCl}_3$ .
- JORISSEN, Bull. acad. roy. sci. Lettres Beaux arts Belg. [3] **3**, 259 (1882). Uses  $\text{KNO}_3$  or  $\text{NaNO}_3$ ,  $\text{HAC}$ , and  $\text{CuSO}_4$  and heats. Red color produced if salicylic acid is present.
- RÉMONT, A., Compt. rend. **95**, No. 18 (1882); Chem. News **46**, 243 (1882). Rapid process for the determination of salicylic acid. Uses  $\text{FeCl}_3$ .
- HEINZELMANN, G., Z. Spiritusind. **7**, 996 (1884); Analyst **10**, 77 (1885). The determination of salicylic acid in urine. Uses  $\text{FeCl}_3$ .
- INCE, W. H., Am. J. Pharm. **59**, 523 (1887). Determines salicylic acid in wines.
- FREHSE, J. pharm. chim. [5] **14**, 507; Z. anal. Chem. **26**, 749 (1887); Chem. News **57**, 262 (1888); J. Soc. Chem. Ind. **6**, 148 (1887). Uses  $\text{FeCl}_3$ .
- ELION, H., Z. anal. Chem. **31**, 96 (1892); Chem. News **65**, 192 (1892). Determination of salicylic acid in beer and similar liquids. Extracts with ether and uses  $\text{FeCl}_3$ .
- SPICA, M., Gazz. chim. ital. **25**, i, 207 (1895). Detection of salicylic acid in wines. Uses  $\text{FeCl}_3$ .
- FREYER, F., Chem.-Ztg. **20**, 820 (1896); Analyst **22**, 39 (1897). Estimation of salicylic acid and its detection in wine, beer, etc. Uses  $\text{FeCl}_3$ .
- FRESENIUS, W. and GRÜNHUT, L., Z. anal. Chem. **38**, 292 (1899); Analyst **25**, 19 (1900). A critical examination of the methods of quantitatively determining salicylic acid. Says  $\text{FeCl}_3$  colorimetric method can only be used when less than 2 mgs. of salicylic acid are present.
- PELLET, H. and GROBERT, S. DE, Bull. Assocn. chim. suc. dist. **20**, 289 (1902); J. Soc. Chem. Ind. **21**, 1416 (1902). Salicylic acid in wines, etc. Use  $\text{H}_2\text{SO}_4$  and  $\text{FeCl}_3$ .
- HARVEY, S., Analyst **28**, 2 (1903); J. Chem. Soc. **84**, ii, 248 (1903). Uses 1 per cent solution of iron alum. Will detect 1 part of salicylic acid in 3,000,000 parts of water.
- MONTANARI, C., Gazz. chim. ital. **34**, i, 290 (1904); J. Chem. Soc. **86**, ii, 522 (1904). Uses  $\text{FeCl}_3$  and also the production of picric acid and action of latter on white wool.
- HARRY, F. T. and MUMMERY, W. R., Analyst **30**, 124 (1905); J. Chem. Soc. **88**, ii, 426 (1905); J. Soc. Chem. Ind. **24**, 514 (1905). Colorimetric estimation of salicylic acid in foodstuffs. Uses  $\text{FeCl}_3$ .
- DUBOIS, W. L., J. Am. Chem. Soc. **28**, 1616 (1906). Determination of salicylic acid in canned tomatoes, catsups, etc. Uses a few drops of 2 per cent ferric alum solution.
- REVIS, C. and PAYNE, G. A., Analyst **32**, 286 (1907). J. Chem. Soc. **92**, ii, 823 (1907). Estimation of salicylic acid in milk and cream. After a lengthy treatment, the usual iron alum method is used. Boric and benzoic acids do not interfere.

- GIBBS, H. D., *J. Am. Chem. Soc.* **30**, 1467 (1908); also in *Philippine J. Sci.* **1908**, with additional data. Separation and determination of salicylic acid and methyl salicylate in foods, etc. Uses method described in U. S. Dept. Agr., Bur. Chem., Bull. **107** (1907), p. 180.
- BIGELOW, W. D., U. S. Dept. Agr., Bull. **122** (1909), p. 64. Estimation of salicylic acid in wine. For small amounts, uses  $\text{FeCl}_3$ .
- SHERMAN, H. C., *J. Ind. Eng. Chem.* **2**, 24 (1910). A source of error in the examination of foods for salicylic acid.  $\text{FeCl}_3$  test not reliable since maltol gives the same violet color as salicylic acid. Jorissen's test is satisfactory. A distinct reddish color is given at a dilution of 1 : 200,000. Gives a number of references to the use of Jorissen's test:  $\text{KNO}_2$  or  $\text{NaNO}_2$ ,  $\text{HAc}$ ,  $\text{CuSO}_4$ , and heat to boiling. Red color produced if salicylic acid is present.
- CASSAL, N. C., *Chem. News* **101**, 289 (1910); *J. Soc. Chem. Ind.* **29**, 835 (1910). Salicylic acid in wines, etc. Uses iron alum.
- SHERMAN, H. S. and GROSS, A., *J. Ind. Eng. Chem.* **3**, 492 (1911); *C. A.* **5**, 2792 (1911); *J. Chem. Soc.* **102**, ii, 395 (1912).  $\text{FeCl}_3$  test simple and delicate but not characteristic. Authors' method detects as little as 0.005 to 0.01 mg. salicylic dissolved in pure water. Uses Na or  $\text{KNO}_2$ ,  $\text{HAc}$ , and  $\text{CuSO}_4$ .
- SCHOTT, F., *Z. Nahr. Genussm.* **22**, 727; *C. A.* **6**, 725 (1912); cf. Sherman and Goss, *C. A.* **5**, 2792. Colorimetric estimation of salicylic acid and copper. The Jorissen reaction is used. 10 mg. salicylic acid per liter give a comparable color. 0.01 mg. Cu may be determined. Sucrose, glucose, lactose, invert sugar and traces of Fe do not interfere, but free mineral acids, tartaric and citric acids, and large amounts of Fe do.
- SAUERLAND, F., *Biochem. Z.* **40**, 67 (1912). Uses ferric alum.
- HEINTZ, W. and LIMPRICH, R., *Z. Nahr. Genussm.* **25**, 706 (1913); *J. Chem. Soc.* **104**, ii, 737 (1913). Detection and estimation of salicylic acid in fruit juices. Use  $\text{FeCl}_3$ .
- SERGER, H., *Z. Nahr. Genussm.* **27**, 319 (1914); *Analyst* **39**, 219 (1914). Estimation of salicylic acid in jams. Use  $\text{FeCl}_3$ .
- THOBURN, T. W. and HANZLIK, P. J., *J. Biol. Chem.* **23**, 163 (1915). The salicylates. II. Methods for the quantitative recovery of salicyl from urine and other body fluids.
- CLAASZ, M., *Arch. Pharm.* **253**, 360 (1915). On the salicylic-ferric chloride reaction. Claims following compound is formed:



Cf. Hantzsch and Desch, *Z. anal. Chem.* **55**, 547 and **49**, 227, who claim the constitution to be:



- ARNY, H. V. and RING, C. H., *J. Ind. Eng. Chem.* **8**, 309 (1916); *C. A.* **10**, 1146 (1916); see also *Proc. 8th Intern. Cong. Appl. Chem.* **26**, 319; cf. Arny and Pickhardt, *Drug. Circ.* **58**, 131 (1914) and *J. Franklin Inst.* Aug. 1915. Color standards and colorimetric assays. Use colored solutions of

- $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to prepare standard color solutions. Use method of Bull. **107**, Bur. of Chem., U. S. Dept. Agr.
- FRESENIUS, W. and GRÜNHUT, L., *Z. anal. Chem.* **60**, 257 (1921); *J. Chem. Soc.* **120**, ii, 602 (1921). Detection and estimation of salicylic acid in wine. Use  $\text{FeCl}_3$ .
- SNELL, F. D., *Colorimetric Analysis*, p. 140, D. Van Nostrand Co., New York, 1921. Determination of salicylic acid by Fehling's solution.

#### Salvarsan.

- KOLLS, A. C. and YOUMANS, J. B., *Bull. Johns Hopkins Hosp.* **34**, 149 (1923); *J. Chem. Soc.* **124**, ii, 800 (1923); *C. A.* **17**, 2433 (1923). A colorimetric method for the estimation of salvarsan in blood and tissues.

#### Selenium.

- MEYER, J., *Z. anorg. Chem.* **34**, 51 (1903). Test for small amounts of selenious acid. Observed that  $\text{Na}_2\text{S}_2\text{O}_4$  produces a red colloidal solution of Se. Very sensitive reaction for Se.
- BRUNCK, O., *Ann.* **336**, 281 (1904). Test for small amounts of selenious acid. Uses  $\text{Na}_2\text{S}_2\text{O}_4$ .
- CLENNELL, J. E., *Eng. Mining J.* **80**, 777 (1905); *Chem.-Ztg. Rep.* **29**, 392 (1905). Adds Na bisulfite to Se in solution as selenite or selenocyanate. Extremely finely divided suspension of Se is produced. Color varies from orange to scarlet, depending upon conditions. Compares against a standard prepared under identical conditions.
- JANNEK, J. and MEYER, J., *Z. anorg. Chem.* **83**, 73 (1913); *J. Chem. Soc.* **104**, ii, 948 (1913). Use Na hyposulfite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) which will detect 0.005 per cent  $\text{SeO}_2$  in water, or 0.002 per cent in concentrated  $\text{H}_2\text{SO}_4$ .
- JANNEK, J. and MEYER, J., *Z. anorg. Chem.* **83**, 75 (1913); *J. Chem. Soc.* **104**, ii, 948 (1913). Use KI and starch. Will detect 0.0005 mg.  $\text{SeO}_2$  in 1 cc. of solution or 0.0025 mg. in concentrated  $\text{H}_2\text{SO}_4$ .
- MEYER, J. and JANNEK, J., *Z. anal. Chem.* **52**, 534 (1913); *J. Chem. Soc.* **104**, ii, 788 (1913). Detection of small quantities of selenious acid. Use  $\text{Na}_2\text{S}_2\text{O}_4$ . Red color due to colloidal Se.
- MEYER, J. and GARN, W. VON, *Z. anal. Chem.* **53**, 29 (1914); *J. Chem. Soc.* **106**, ii, 67 (1914); *J. Soc. Chem. Ind.* **33**, 21 (1914). Detection and estimation of minute traces of selenious acid. Use gum arabic, HCl, and KI. Color due to I and colloidal Se.  $\text{H}_2\text{SeO}_3 + 4\text{HI} \rightarrow 2\text{I}_2 + \text{Se} + 3\text{H}_2\text{O}$ .
- SCHMIDT, E., *Arch. Pharm.* **252**, 161 (1914); *J. Chem. Soc.* **106**, ii, 672 (1914). Detection of very small quantities of selenious acid in sulfuric acid. Uses codeine phosphate. Bluish-green coloration after 15 minutes. Will detect 0.0005 per cent selenious acid.
- SNELL, F. D., *Colorimetric Analysis*, p. 138, D. Van Nostrand Co., New York, 1921. Determination of selenium as selenious acid by KI.

#### Sensitiveness.

- FOLKARD, C. W., *Chem. News* **75**, 73 (1897). Note on the limit of accuracy attainable in colorimetry.



- HORN, D. W., Am. Chem. J. **35**, 253 (1906). Variable sensitiveness in the colorimetry of chromium. I. Sensitiveness in color matching of  $\text{CrO}_4^{--}$  at a maximum at concentrations between 0.004 N and 0.008 N.
- HORN, D. W. and BLAKE (Miss) SUE A., Am. Chem. J. **36**, 195 (1906). Variable sensitiveness in colorimetry. II. This is the second of a series of three papers (Horn, Am. Chem. J. 35, 253; Horn and Blak, *ibid.*, **36**, 195, 516) on the variable sensitiveness in colorimetry. The authors show that with equal depths "at certain definite concentrations, the comparisons in the colorimetric determination of chromium can be made with greater ease and accuracy than at other concentrations" and it is held by them that this relation "is a perfectly general one throughout colorimetry."
- HORN, D. W. and BLAKE (Miss) SUE A., Am. Chem. J. **36**, 516 (1906); C. A. **1**, 156 (1907). Variable sensitiveness in colorimetry. III. Cu in ammoniacal  $\text{CuSO}_4$  solutions. With 0.637 mg. Cu in 50 cc., 0.0008 mg. Cu makes a perceptible difference of color in the 50 cc. solution. A slight change in concentration in either direction causes the sensitiveness to decrease rapidly.
- WELLS, R. C., J. Am. Chem. Soc. **33**, 504 (1911). Sensitiveness of the colorimetric estimation of titanium.
- LEROY, G., Ann. fals. **10**, 208 (1917); J. Chem. Soc. **112**, ii, 418 (1917). Method of rendering more sensitive colorimetric analyses. When the amount of constituent present is extremely small, add a known amount to bring the concentration within the best limits for comparison.

### Silica.

- JOLLES, A. and NEURATH, F., Z. angew. Chem. **1898**, 315; J. Chem. Soc. **74**, ii, 455 (1898). Colorimetric estimation of silica in water. Method based upon the yellow coloration produced by heating silicic acid solutions with a  $\text{HNO}_3$  solution of molybdic acid.  $\text{PO}_4$  in waters is usually absent or so small as to be insignificant.
- SALVADORI, R. and PELLINI, G., Gazz. chim. ital. **30**, i, 191 (1900); J. Chem. Soc. **78**, ii, 367 (1900). Colorimetric method for the estimation of silica in mineral waters. Uses molybdic acid in  $\text{HNO}_3$ . Same as the method of Jolles and Neurath (Z. angew. Chem. **1898**, 315) which was perfected by Woodman and Cayvan [J. Am. Chem. Soc. **23**, 96 (1901)].
- WOODMAN, A. G. and CAYVAN, L. L., J. Am. Chem. Soc. **23**, 96 (1901). The determination of phosphate in potable waters. Study the effect of  $\text{SiO}_2$  on the  $\text{NH}_4$  molybdate- $\text{HNO}_3$  method for  $\text{PO}_4$ .
- VEITCH, F. P., J. Am. Chem. Soc. **25**, 169 (1903). On the colorimetric determination of small quantities of phosphoric acid and of silica. Uses  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ . Both  $\text{PO}_4$  and  $\text{SiO}_2$  give a yellow coloration but there is a difference in the rate of reaching the maximum intensity of color.
- SCHREINER, O., J. Am. Chem. Soc. **25**, 1056 (1903). Uses  $\text{NH}_4$  molybdate and  $\text{HNO}_3$ .
- WINKLER, L. W., Z. angew. Chem. **27**, 511 (1914); J. Chem. Soc. **108**, ii, 373 (1915). Estimation of silicic acid in natural waters. Uses  $\text{NH}_4$  molybdate and  $\text{HCl}$ .

- SNELL, F. D., *Colorimetric Analysis*, p. 124, D. Van Nostrand Co., New York, 1921. Determination of silica by ammonium molybdate in the presence of phosphorus.
- DIÉNERT, F. and WANDENBULCKE, F., *Compt. rend.* **176**, 1478 (1923); *J. Chem. Soc.* **124**, ii, 507 (1923). The estimation of silica in waters. Based upon the yellow coloration produced by  $\text{NH}_4$  molybdate with silica in solution. Since silica in colloidal suspension does not give the molybdate reaction, colloidal and non-colloidal silica may thus be differentiated.
- ISAACS, M. L., *Bull. soc. chim. biol.* **6**, 157 (1924). Colorimetric determination of silicon in tissues. Used  $\text{NH}_4$  molybdate and reduced the resulting silicomolybdate to a deep blue compound by the addition of sodium sulfite. Was able to determine as little as 0.5 mg. of Si in 100 g. of dried tissue. Since only a 0.5 g. sample of tissue was used for ashing, Isaacs' colored solution contained only 0.0025 mg. of  $\text{SiO}_2$  or 0.00115 mg. of Si.
- BERTRAND, *Bull. soc. chim. biol.* **6**, 656 (1924). Determination of silicon in animal tissues. Uses Isaacs' sodium sulfite method and severely criticises it. Says under the same conditions of this method phosphate would give a phosphomolybdate and that this also would be reduced by sodium sulfite to give a blue colored solution.
- ATKINS, W. R. G. and WILSON, E. G., *Biochem. J.* **20**, 1223 (1926); *C. A.* **21**, 1778 (1927). Colorimetric estimation of minute amounts of compounds of silicon, of phosphorus and of arsenic.
- FOULGER, J. H., *J. Am. Chem. Soc.* **49**, 429 (1927). Colorimetric determination of silicon in tissues by Isaacs' method. Confirms Isaacs' work, *Bull. soc. chim. biol.* **6**, 157 (1924). Shows that "phosphomolybdates do not give a blue reduction product when treated with sodium sulfite in the presence of acetic acid," and that "quantitative mixtures of silicate and phosphate do not give a color more intense than would be given by solutions having the same concentration of silicate but no phosphate."

Silicon. (See Silica.)

#### Silver.

- WHITBY, G. S., *Z. anorg. Chem.* **67**, 62 (1910); *J. Chem. Soc.* **98**, ii, 654 (1910); 7th Intern. Congr. Appl. Chem. (London), **1909**; *J. Soc. Chem. Ind.* **28**, 749 (1909). The detection and estimation of very small quantities of silver. Method based upon formation of colloidal silver by several organic compounds (such as sucrose, starch, dextrin, cellulose, and glycerol) added to Ag salts in NaOH solution. Dark colored solution formed. 0.000002 g. of Ag in 50 cc. may be detected. Ammonia must be absent, but Cu, Zn, Hg, Bi, Cd, and Pb have no influence if not present in sufficient quantity to produce a visible precipitate with the NaOH.

#### Skatole.

- HERTER, C. A. and FOSTER (Miss) M. L., *J. Biol. Chem.* **2**, 267 (1906-7). On the separation of indole from skatole and their quantitative determination.

Use  $\beta$ -naphthaquinone-sodium-mono-sulfonate for indole. Use Ehrlich's aldehyde reagent for skatole after separation.

NELSON, V. E., J. Biol. Chem. **24**, 527 (1916). Some color reactions for indole and skatole. Possibility of some quantitative methods.

### Sodium.

DOISY, E. A. and BELL, R. D., J. Biol. Chem. **45**, 313 (1920-21). The determination of sodium in blood. Use alkaline tartrate, sulfanilic acid and  $\alpha$ -naphthylamine.

DOISY, E. A. and BELL, R. D., J. Biol. Chem. **45**, 319 (1920-21). Colorimetric determination of sodium in blood.

YOSHIMATSU, S., Tōhoku J. Exptl. Med. **8**, 496 (1927). Colorimetric determination of sodium in 0.1 cc. of serum or blood. "The method involves 4 steps: (1) pptn. of Na as pyroantimonate, (2) sepn. of the supernatant liquid by the centrifuge, (3) dissolving the pyroantimonate in HCl, and (4) the conversion of the Sb to the sulfide by the addn. of  $\text{Na}_2\text{S}$ , when the orange red color produced is read colorimetrically. The av. deviation of this method from Kramer's method was  $\pm 3.0\%$ ." L. W. Riggs, C. A. **21**, 2145 (1927).

### Soil Toxicity.

CARR, R. H., J. Ind. Eng. Chem. **13**, 931 (1921). Measuring soil toxicity, acidity, and basicity. The soil is extracted with alcoholic thiocyanate solution and the extract treated with a few drops of logwood tincture.

### Standards.

JACKSON, D. D., Tech. Quart. **13**, 314 (1900). Permanent standards for use in the analysis of water.

KENDALL, L. M., Tech. Quart. **17**, 277 (1904). Permanent standards in water analysis.

LOVIBOND, J. W., J. Soc. Chem. Ind. **28**, 500 (1909); C. A. **3**, 2278 (1909). Some requirements of a color standard.

ARNY, H. V., Am. Druggist **60**, 35 (1912). Color standards.

ARNY, H. V., Proc. 8th Intern. Cong. Appl. Chem. **26**, 319 (1912). International standards for colored fluids.

ARNY, H. V. and PICKHARDT, E. G., Drug. Circ. **58**, 131 (1914); C. A. **8**, 2540 (1914); see also J. Franklin Inst. Aug. **1915**. The problem of color standardization. Develop a supplemental set of colors ( $\text{CoCl}_2 \cdot 5\text{NH}_3 \cdot \text{H}_2\text{O}$ ,  $(\text{NH}_4)_2\text{Cr}_2\text{O}_7$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) to furnish the needed blues and some of the reds.

ARNY, H. V. and RING, C. H., J. Franklin Inst. **180**, 199 (1915); C. A. **9**, 2492 (1915). Standardized colored fluids. A continuation of the work reported in Drug. Circ. **58**, 131.

ARNY, H. V. and RING, C. H., J. Am. Pharm. Assocn. **4**, 1294 (1915); C. A. **10**, 804 (1916). A further report on the work recorded in J. Franklin Inst. **180**, 199.

ARNY, H. V. and RING, C. H., J. Ind. Eng. Chem. **8**, 309 (1916); C. A. **10**, 1146 (1916); see also Proc. 8th Intern. Cong. Appl. Chem. **26**, 319; cf. Arny and Pickhardt, Drug. Circ. **58**, 131 (1914) and J. Franklin Inst. Aug. **1915**. Color

- standards and colorimetric assays. Use colored solutions of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to prepare standard color solutions. Following determinations are dealt with in detail:  $\text{NH}_3$  (Nessler's reagent);  $\text{NO}_3$  (phenolsulfonic acid);  $\text{NO}_2$  (sulfanilic acid and naphthylamine hydrochloride); vanillin (method in Bull. **107**, Bur. Chem., U. S. Dept. Agr. and also the Folin and Denis method, J. Ind. Eng. Chem. **4**, 670 (1912); uric acid (phosphomolybdic acid); salicylic acid (method of Bull. **107**, Bur. Chem. U. S. Dept. Agr.); phosphates (ammonium molybdate reagent).
- TAKATA, M., Tōhoku J. Exptl. Med. **1920**, **1**, 460; C. A. **15**, 2457 (1921); Analyst **47**, 32 (1922). Use of dyes as standards in colorimetric methods.
- SONDÉN, K., Arkiv. Kemi, Mineral. Geol. **8**, No. 7, 10 pp. (1921); C. A. **16**, 2090 (1922); J. Chem. Soc. **122**, ii, 862 (1922); J. Soc. Chem. Ind. **41**, 962A (1922). The use of colored glasses in place of liquids in colorimetric investigations.
- MELLON, M. G., Proc. Indiana Acad. Sci. **1922**, 164; C. A. **18**, 1446 (1924). The use of solutions of inorganic salts as permanent color standards.
- YOE, J. H. and EDGAR, G., J. Phys. Chem. **27**, 65 (1923). Use standardized cobalt blue glasses as color standards in determining the amount of an indanthrene dye (Ponsol Yellow G) reduced by alkaline  $\text{Na}_2\text{S}_2\text{O}_4$ .
- HAHN, F. V. von, Z. angew. Chem. **36**, 366 (1923); J. Chem. Soc. **124**, ii, 657 (1923); J. Soc. Chem. Ind. **42**, 866A (1923). Colorimetric methods with the aid of Wilhelm Ostwald's color standards.
- ARNY, H. V. and TAUB, A., J. Am. Pharm. Assocn. **12**, 839 (1923); C. A. **18**, 1033 (1924); J. Franklin Inst. **196**, 858 (1923). Standardized colored fluids and some official colorimetric tests.
- HARRISON, A. P., Science **57**, 716 (1923). Note on preparing color standards.
- WHIPPLE, M. C., Eng. Contr. **62**, 80 (1924); C. A. **18**, 3242 (1924). Permanent standards for iron determinations. Shows that the cobalt chloride usually purchased varies in composition. Should be  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ .
- YOE, J. H., J. Phys. Chem. **28**, 1211 (1924). Uses standardized cobalt blue glasses as color standards in determining the amount of certain vat dyes (Ponsol Blue G, Ponsol Blue R, Ponsol Dark Blue BR, and Ponsol Violet RR) reduced by alkaline  $\text{Na}_2\text{S}_2\text{O}_4$ .

### Starch.

- AMBÜHL, G., Chem.-Ztg. **19**, 1508 (1895).
- DENNSTEDT, M. and VOIGTLÄNDER, F., Forschungs. u. Lebensmittel **2**, 173 (1895).
- CASSEL, C., Z. Spiritusind. **35**, 591 (1912); Chem. Zentr. **64** (1913). Colorimetric Method for determination of starch.
- THOMAS, W., J. Am. Chem. Soc. **46**, 1670 (1924); C. A. **18**, 2538 (1924). Determination of starch and other "reserve" carbohydrates in plants. Use picric acid.

### Stercobilin.

- GOFFON, R., Compt. rend. soc. biol. **83**, 60 (1920); from J. pharm. chim. **21**, 286 (1920); J. Chem. Soc. **118**, ii, 399 (1920); C. A. **14**, 3256 (1920). Uses a saturated solution of  $\text{HgCl}_2$  and ammonia. Red color obtained.

BORRIEN, V., *Compt. rend. soc. biol.* **83**, 211 (1920); *J. Chem. Soc.* **118**, ii, 520 (1920); *C. A.* **16**, 1442 (1922). Goiffon's colorimetric method for the estimation of stercobilin. Accurate estimation only possible when the substance has been isolated chemically pure. No method at present will do this.

### **Strychnine.**

SCANDOLA, E., *Chem. Zentr.* **1911**, I, 593; from a reprint from *Boll. Soc. Med. Chirurg. di Pavia*, **1910**; *J. Chem. Soc.* **100**, ii, 553 (1911). Uses Mandelin's reagent ( $\text{NH}_4$  vanadate dissolved in  $\text{H}_2\text{SO}_4$ ). Solution should not contain any other alkaloid but strychnine.

### **Sugar.** (See also **Carbohydrate, Dextrose, Glucose, and Lactose.**)

PAVEN, Dingler's polytech. J. **27**, 372 (1828); ref. given in *Z. anal. Chem.* **6**, 424 (1866). Color of raw sugar, saps, and syrup in the manufacture of sugar; also the decolorizing power of charcoal.

JOHNSON, G., *Brit. med. J.* **1883**, 504. On picric acid as a test for albumen and sugar in the urine. Uses picric acid and KOH.

MACLEOD, J. J. R., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.* **4**, xvii (1908). A comparison of W. Reid's and Schenck's methods for the estimation of sugar in blood.

FORAILONG, R., *Bull. assocn. chim. suc. dist.* **27**, 1188; *C. A.* **4**, 2584 (1910). Colorimetric method for the approximate determination of small quantities of sugar by means of  $\alpha$ -naphthol.

WACKER, L., *Sitzb. Phys.-Med. Ges. Würzburg*, **1910**; *Zentr. Biochem. Biophys.* **11**, 487; *C. A.* **5**, 3086 (1911). Colorimetric method for determining the sugar in blood.

WACKER, L., *Z. physiol. Chem.* **67**, 197 (1910); *C. A.* **5**, 1473 (1911). A colorimetric method for the determination of sugar in the blood. Proposes the use of *p*-phenylhydrazinesulfonic acid and NaOH.

FORSCHBACH and SEVERIN, *Zentr. ges. Physiol. Path. Stoffw.* [n. s.] **6**, 54 (1911); *C. A.* **5**, 2264 (1911). Colorimetric estimation of blood sugar. I. The estimation of blood carbohydrates by Wacker.

FORSCHBACH and SEVERIN, *Zentr. ges. Physiol. Path. Stoffw.* **6**, 177 (1911); *C. A.* **5**, 2392 (1911). Colorimetric determination of sugars in the blood.

WACKER, L., *Zentr. ges. Physiol. Path. Stoffw.* **6**, 524; *C. A.* **5**, 3691 (1911). Colorimetric determination of sugar in the blood. A response to Forschbach and Severin. W. compares his method with that of F. and S. and finds the results do not agree.

FORSCHBACH and SEVERIN, *Zentr. ges. Physiol. Path. Stoffw.* **6**, 665 (1911); *C. A.* **5**, 3691 (1911). The application of the Autenrieth-Tesdorpf method of sugar determination in urine to the quantitative determination of sugar in the blood.

AUTENRIETH, W. and MÜLLER, G., *Münch. med. Wochschr.* **63**, 899 (1911).

UNDERHILL, F. P. and FINE, M. S., *J. Biol. Chem.* **10**, 271 (1911-12). Studies in carbohydrate metabolism. II. The prevention and inhibition of pancreatic diabetes. Estimate sugar in urine with a Schmidt and Haensch triple shadow saccharimeter.

- AUTENRIETH, W. and FUNK, A., Münch. med. Wochschr. **59**, 689 (1912).
- ESCHLE, O., Fortschritte Med. **30**, 326 (1912).
- REICHER, K. and STEIN, E. H., Z. exptl. Path. Therap. **10**, 533; C. A. **7**, 619 (1913). Present a defense of their colorimetric method of estimating blood and tissue sugar, based on the  $\alpha$ -naphthol reaction empirically controlled.
- JÄRVINEN, K. K., Z. anal. Chem. **52**, 14 (1913). A quantitative method for determining sugar in urine. Make use of the Almén-Nylander reagent (Z. anal. Chem. **9**, 494; Z. Physiol. Chem. **50**, 36; Biochem. Z. **16**, 489).
- LEWIS, R. C. and BENEDICT, S. R., Proc. Soc. Exptl. Biol. Med. **11**, 57 (1913-14). A method for the estimation of sugar in small quantities of blood.
- EPSTEIN, A. A., J. Am. Med. Assocn. **63**, 1667 (1914). Blood sugar. Uses the Sahli-Gower hemoglobinometer. Modifies the method of Lewis and Benedict.
- RIEGLER, F., Z. anal. Chem. **53**, 245 (1914). Colorimetric estimation of sugar in urine. Uses a Cu solution.  $\text{Cu}_2\text{O}$  precipitated by the sugar and Cu estimated colorimetrically.
- AUTENRIETH, W. and MONTIGNY, W., Münch. med. Wochschr. **61**, 1671 (1914); Kolloid-Z. **21**, 41 (1917); C. A. **9**, 213 (1915). Determination of sugar in blood.
- SHAFFER, P. A., J. Biol. Chem. **19**, 285 (1914). On the determination of sugar in blood. Colorimetric determination of cuprous oxide.
- LEWIS, R. C. and BENEDICT, S. R., J. Biol. Chem. **20**, 61 (1915). A method for the estimation of sugar in small quantities of blood. Based upon the red color (probably picramic acid) obtained by heating a dextrose solution with picric acid and  $\text{Na}_2\text{CO}_3$ .
- SCHAFFER, F., Jahresversamml. Schweiz. Vereins. anal. Chem., Zurich, June 5, 1915; through J. Soc. Chem. Ind. **34**, 1025 (1915). Colorimetric determination of pentoses and methylpentoses in wine.
- PEARCE, R. G., J. Biol. Chem. **22**, 525 (1915). A criticism of the Bang and Lewis-Benedict methods for the estimation of blood sugar, with suggestions for a modification of the latter method. Details on pp. 531-32.
- BERNHARD, A., Diss. Brooklyn Polytech. Inst. June 1915; Sugar **17**, No. 11, 41 (1915); C. A. **10**, 1230 (1916). A simple colorimetric method for the determination of free reducing sugar and total carbohydrates in miscellaneous food materials. Uses the color reaction produced by heating dextrose in alkaline solution with picric acid. Picramic acid formed.
- MCDANELL, L., J. Lab. Clin. Med. **1**, 804 (1915-16). The estimation of sugar in the blood by the Lewis-Benedict (J. Biol. Chem. **20**, 61) method. The method is based on the formation of a red color due to the reduction of picric acid to picramic acid by dextrose, in an alkaline medium.
- MACLEOD, J. J. R., J. Lab. Clin. Med. **1**, 445 (1915-16). A rapid and accurate clinical method for the estimation of sugar in small quantities of blood.
- EPSTEIN, A. A. and BAEHR, G., J. Biol. Chem. **24**, 1 (1916). Use a modification of the Lewis and Benedict method.
- EPSTEIN, A. A. and BAEHR, G., J. Biol. Chem. **24**, 17 (1916). Blood sugar determined by Epstein's method.

- MYERS, V. C. and BAILEY, C. V., *J. Biol. Chem.* **24**, 147 (1916). The Lewis and Benedict method for the estimation of blood sugar, with some observations obtained in disease.
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#### Sulfur.

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- NASKE, T., *Stahl u. Eisen* **22**, 333 (1902); *Analyst* **27**, 206 (1902); *J. Iron Steel Inst. London* **61**, 649 (1902); *Chem.-Ztg.* **26**, 333 (1902); *J. Soc. Chem. Ind.*

- 21**, 563 (1902). Colorimetrische Methode zur Bestimmung des Schwefels in Roheisen. "Uses paradimethylphenyldiamine which forms colored tetramethylthyoninchloride; results not good. NaOH is not a good absorbent as  $\text{Na}_2\text{S}$ ,  $\text{NaHS}$  and polysulfides are formed; air converts these partly to thiosulfate; some S goes to  $\text{H}_2\text{SO}_4$ , some  $\text{H}_2\text{S}$  escapes; longer action of passing gases leaves only NaOH in flask. This method (of Lindsay and Naske) gives only qualitative test for S." H. B. Pulsifer: "The Determination of Sulfur in Iron and Steel," Chemical Publishing Co., Easton, Pa., 1922.
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that different substances of the class in question have different H-ion concns. within which the violet color is formed to the best advantage and unless precautions are taken to maintain the proper H-ion concn., 2 solns. with equal content of pyrogallol or catechol nucleus may not give the same intensity of color in the test. Thus with the following substances the color was produced within these pH limits; pyrogallol 6.5-10.3, gallic acid 5.9-10.3, tannic acid 4.1-11.1, catechol 7.0-10.3, pyrocatechuic acid 6.3-10.4. In applying the test to pyrogallol derivs. it is best to modify the reagent by adding  $\text{NH}_4\text{OAc}$  soln. to act as a buffer salt and with catechol a few drops of  $\text{NH}_4\text{OH}$  should be used." W. T. H., C. A. **19**, 2620 (1925).

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PHILIP, J. C. and BRAMLEY, A., Proc. Chem. Soc. April 17, **1913**; Chem. News **107**, 226 (1913). The reaction between ferric salts and thiocyanates.

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- WELLER, A., *Ber.* **15**, 2592 (1882). On the test and determination of titanium. Uses  $H_2O_2$ . A yellow or orange colored solution is obtained.
- JACKSON, E., *Chem. News* **47**, 157 (1883). Ti in HCl or  $H_2SO_4$  gives a yellow or orange color when  $H_2O_2$  is added, the depth of color depending upon the conc. Ti. Converse of the test may be used to test for  $H_2O_2$ . Suggests the method may be made quantitative.
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- DUNNINGTON, F. P., *J. Am. Chem. Soc.* **13**, 210 (1891). On metatitanic acid and the estimation of titanium by hydrogen peroxide. Points out the necessity for the presence of at least 5 per cent of  $H_2SO_4$  in solutions to be treated with  $H_2O_2$ . Believed this necessary to prevent partial reversion to metatitanic acid, which does not give a color with  $H_2O_2$ .
- HILLEBRAND, W. F., *J. Am. Chem. Soc.* **17**, 718 (1895); *Chem. News* **72**, 158 (1895). Points out that it is imperative to use only  $H_2O_2$  which is free from HF or fluoride, since fluorine bleaches the  $TiSO_4-H_2O_2$  color to a marked degree.
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- FABER, P., Chem.-Ztg. **31**, 263 (1907); Z. anal. Chem. **46**, 277 (1907); C. A. **1**, 1528 (1907). The colorimetric determination of titanium and its application in the presence of iron. Uses  $\text{H}_2\text{O}_2$  in  $\text{H}_2\text{SO}_4$ -Ti solutions. Method accurate in the presence of large quantities of iron if a measured excess of  $\text{H}_3\text{PO}_4$  is added both to standard titanium solution and the test solution. This modified Weller's method dissolves HAc mass, after the K bisulfate fusion, in dilute  $\text{H}_2\text{SO}_4$  (sp. gr. 1.10 to 1.12) heated to boiling instead of cold water which requires 36 hours at least. The excess acid prevents formation of any  $\text{TiOSO}_4$  which does not color solution so intensely as  $\text{Ti}(\text{SO}_4)_2$  when the  $\text{H}_2\text{O}_2$  is added. See, however, Dunnington, J. Am. Chem. Soc. **13**, 210 (1891).
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- to about 1.5 mg.  $\text{TiO}_2$  per 100 cc. Change in concentration required to show a perceptible difference in color intensity between 2 solutions at favorable concentrations was found to be about 6.5 per cent. This is about the same as results of others for Cr and Cu solutions. With suitable precautions, such as comparing by substitution and taking mean of several settings or of the two perceptible different extremes, accuracy appears to be about 2 per cent.
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- MOLSTAD, R., Tids. Kemi Farm. Terapi **11**, 313 (1914); C. A. **9**, 422 (1915). Sample and standard rutile each dissolved in a mixture of  $\text{H}_2\text{O}$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_3\text{PO}_4$  and oxidized with  $\text{H}_2\text{O}_2$ .
- HILLEBRAND, W. F., U. S. Geol. Survey Bull. **700** (1919), p. 155. Uses  $\text{H}_2\text{O}_2$ . Titanium solution must contain at least 5 per cent  $\text{H}_2\text{SO}_4$ . Gives principle of the method, conditions that affect it, and details of the procedure.
- TREADWELL, F. P. and HALL, W. T. (Translator from the German), Analytical Chemistry. Vol. II. Quantitative Analysis. 5 ed., p. 100, John Wiley & Sons, Inc., New York, **1919**. Uses  $\text{H}_2\text{O}_2$ .
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**Vanadium.**

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- pounds in the higher form of oxidation ( $V_2O_5$ ) give a violet coloration which gradually changes to orange." A delicate test for V, which is not interfered with by Ti, Mo, W, or Al, but if Fe is present it must be removed by fusion with  $Na_2CO_3$ . In a test analysis, a solution containing 0.0056 g. V in a mixture with Mo, W, and Fe, gave 0.0055 g. V.
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- KROPP, A., Z. angew. Chem. **35**, 366 (1922); C. A. **16**, 3604 (1922); J. Chem. Soc. **122**, ii, 590 (1922); J. Soc. Chem. Ind. **41**, 594A (1922). The colorimetric determination of vanadium in steel. Uses  $H_2O_2$  on acid solution of V.  $H_3PO_4$  used to repress color due to  $Fe^{+++}$  and W. Carbon removed by oxidation with  $(NH_4)_2S_2O_8$ . If Ni or Cr in sample, same amounts of them are added to the standard.

MISSON, G., *Bull. soc. chim. Belg.* **31**, 123 (1922); *J. Chem. Soc.* **122**, ii, 459 (1922); *Analyst* **47**, 321 (1922). Detection and estimation of vanadium in steels. Uses  $\text{Na}_2\text{O}_2$  in dilute  $\text{HNO}_3$ .

#### Vanillin.

MOERK, F., *Am. J. Pharm.* **63**, 521 (1891). Uses a ferrous salt and oxidizes it with bromine water. This method will detect 1 part of vanillin in 100,000 instead of 1 part in 2,000 when  $\text{FeCl}_3$  is used.

MOERK, F., *Am. J. Pharm.* **63**, 572 (1891); *J. Soc. Chem. Ind.* **11**, 637 (1892); cf. U. S. Dept. Agr. Bur. Chem. Bull. **107**, p. 157. Colorimetric estimation of vanillin in vanilla extracts. Uses a ferrous salt and oxidizes it with bromine water.

DIETERICH, K., *Z. anal. Chem.* **37**, 453 (1898).

HUBBARD, W. S., *J. Ind. Eng. Chem.* **4**, 669 (1912); *J. Chem. Soc.* **104**, ii, 448 (1913). Difficulties in the colorimetric estimation of vanillin. Points out various disadvantages in the Br-water and  $\text{FeSO}_4$  method.

FOLIN, O. and DENIS, W., *J. Ind. Eng. Chem.* **4**, 670 (1912). A new colorimetric method for the determination of vanillin in flavoring extracts. Use phosphotungstic-phosphomolybdic reagent.

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ARNY, H. V. and RING, C. H., *J. Ind. Eng. Chem.* **8**, 309 (1916); *C. A.* **10**, 1146 (1916); see also *Proc. 8th Intern. Cong. Appl. Chem.* **26**, 319; cf. Arny and Pickhardt, *Drug. Circ.* **58**, 131 (1914) and *J. Franklin Inst.* Aug. **1915**. Use colored solutions of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to prepare standard color solutions. Determine vanillin by method in Bull. **107**, Bur. Chem., U. S. Dept. Agr. and also Folin and Denis method, *J. Ind. Eng. Chem.* **4**, 670 (1912).

ESTES, C., *J. Ind. Eng. Chem.* **9**, 142 (1917); *J. Chem. Soc.* **112**, ii, 343 (1917). New qualitative test and colorimetric method for the estimation of vanillin. Uses an acid- $\text{Hg}(\text{NO}_3)_2$  reagent. Produces a violet-red color.

#### Water.

RICHARDS, (Miss) E. H. and ELLMS, J. W., *J. Am. Chem. Soc.* **18**, 68 (1896). The coloring matter of natural waters.

TRILLAT, A., *Compt. rend.* **162**, 486 (1916); *J. Chem. Soc.* **110**, ii, 269 (1916). A colorimetric method used by the Romans to characterize soft waters. The method of the Greeks and Romans (compare Hippocrates, "Traité des Aïrs, des Eaux et des Lieux," Chapter 36) for detecting the presence of alkalis in natural waters by the decoloration of red wine can be made to give an approximate measure of the hardness of different waters. The degree of hardness is indicated by the number of drops of red wine required to produce a coloration in the water as compared against a standard water.

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- SNELL, F. D., *Colorimetric Analysis*, p. 144, D. Van Nostrand Co., New York, 1921. Determination of the color of water.
- KOLTHOFF, I. M., *Pharm. Weekblad* **60**, 227 (1923); *C. A.* **17**, 1774 (1923); *J. Chem. Soc.* **124**, ii, 248 (1923). Colorimetric determination of the water content of absolute alcohol. Based upon the fact that the sensitivity of azo indicators, e.g., M. O., to acid diminishes with increasing the EtOH concentration of the medium.
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#### Wood Fiber.

- VALENTA, E., *Chem.-Ztg.* **42**, 503 (1904); *Analyst* **29**, 289 (1904). A colorimetric method for the determination of wood fiber in paper.

#### Xanthophyll.

- PALMER, L. S., *J. Biol. Chem.* **23**, 261 (1915). Xanthophyll, the principal natural yellow pigment of the egg yolk, body fat, and blood serum of the hen. The physiological relation of the pigment to the xanthophyll of plants.

#### Zinc.

- COOPER, A. J., *J. Soc. Chem. Ind.* **5**, 84 (1886). Note on the detection of metals in drinking water. Gives a table showing the delicacy of the following tests:  $K_4Fe(CN)_6$ ,  $NH_4OH$ , and  $H_2S$  tests for Cu;  $(NH_4)_2S$  test for Zn;  $H_2S$  test for As;  $K_2CrO_4$  and  $H_2S$  tests for Pb.
- CAMPO Y Cerdan, A DEL, *Ann. chim. anal. appl.* **14**, 205; *C. A.* **3**, 2785 (1909).  $NH_4HO$  added till  $Zn(OH)_2$  redissolves, then add 1 cc. of an alcohol or ethereal solution of resorcinol. A blue color appears whose depth depends upon the concentration of Zn. Sensitive to 1 part in 100,000. Ca gives a green color, Ni a blue, and Co a red. Hence, these metals must be removed.
- CAMPO Y Cerdan, A DEL, *Anales soc. españ. fis. quim.* **7**, 63 (1909); *J. Chem. Soc.* **96**, ii, 439 (1909). Color test for zinc salts. States that 0.005 mg. of Zn in 1 cc. may be detected by the blue coloration produced on addition of ammonia and resorcinol. About 1 hr. required for color to develop at this low concentration. Cd salts produce a green color with resorcinol and Cu salts a black precipitate.
- WINKLER, L. W., *Z. angew. Chem.* **26**, 38 (1913); *J. Chem. Soc.* **104**, ii, 246 (1913); *J. Soc. Chem. Ind.* **32**, 157 (1913). Detection and colorimetric estimation of lead, copper, and zinc in potable water. Uses  $Na_2S$  for Pb;  $K_4Fe(CN)_6$  and  $KHCO_3$  for Cu; and a turbidity method for Zn.
- COMPO Y Cerdan, A DEL, and DE LA PUENTE, J., *Anales soc. españ. fis. quim.* **11**, 98 (1913); *C. A.* **7**, 3291 (1913). Colorimetric determination of traces of zinc. Zn gives blue color with  $NH_4OH$  and resorcinol. Limits of concentrated 0.041 g. to 0.00018 g. Zn per cubic centimeter. Errors observed for concentrations of Zn 0.1 to 3.2 mg. per 100 cc., varied from 0.06 to 6.6

- per cent. Solution is altered by air and must be protected with liquid paraffin until ready for examination.
- MELDRUM, R., *Chem. News* **116**, 271, 295, 308 (1917). The identification and estimation of zinc in water. Uses  $(\text{NH}_4)_2\text{S}$  and  $\text{K}_4\text{Fe}(\text{CN})_6$  methods.
- BIRCKNER, V., *J. Biol. Chem.* **38**, 191 (1919). The zinc content of some food products. Turbidimetric estimation of pot. zinc ferrocyanide.
- BODANSKY, M., *J. Biol. Chem.* **44**, 399 (1920). Biochemical studies on marine organisms. II. The occurrence of zinc. Turbidimetric.
- BODANSKY, M., *J. Ind. Eng. Chem.* **13**, 696 (1921); *C. A.* **15**, 3428 (1921); *Analyst* **46**, 518 (1921); *J. Soc. Chem. Ind.* **40**, 829A (1921). The determination of small quantities of zinc. Uses  $\text{K}_4\text{Fe}(\text{CN})_6$ . A turbidimetric method.
- SNELL, F. D., *Colorimetric Analysis*, p. 81, D. Van Nostrand Co., New York, **1921**. Determination of zinc by resorcinol.
- JÄRVINEN, K. K., *Z. Nahr. Genussm.* **45**, 183 (1923); *J. Chem. Soc.* **124**, ii, 655 (1923). Colorimetric estimation of small quantities of metals in food stuffs and the preliminary destruction of the organic matter. Details for the destruction of the organic matter are given and for the estimation of Sn and Pb in the presence of one another, Cu and Zn in the presence of one another, Al, Ni, As, and Sb. Uses  $\text{H}_2\text{S}$  or  $\text{Na}_2\text{S}$ .
- LUTZ, R. E., *J. Ind. Hyg.* **7**, 273 (1925); *C. A.* **19**, 2614 (1925). Determination of a small amount of zinc in organic materials. Uses urobilin. 0.1–0.5 mg. Zn can be determined with an accuracy of 10 per cent.
- SCOTT, W. W., *Standard Methods of Chemical Analysis*, 4 ed., p. 607, D. Van Nostrand Co., New York, 1925. A turbidity method. The Zn is precipitated as  $\text{ZnS}$ , the latter dissolved in  $\text{HCl}$  and ferrocyanide added. The suspension thus formed is compared with a standard.

# PART VI

## TABLES

### ANALYSES OF CHEMICAL GLASSWARE<sup>1</sup>

The marks on both beakers and flasks were identical in the case of all the wares examined except Jena, in which an "N" appeared below the main body of the trade mark on the flasks but did not appear on the beakers. Therefore, with the Jena ware analyses were made of both beakers and flasks, but with the other wares the flasks were not analyzed. It is evident from the results that there is no difference in composition between the Jena beakers and flasks. Table XLIV shows the analyses of the wares tested by the Bureau of Standards.

TABLE XLIV

Ware	Kavalier beaker	M. E. G. Co. beaker	Pyrex beaker	Jena beaker	Jena flask	Nonsol beaker	Fry beaker	Libbey beaker
Al <sub>2</sub> O <sub>3</sub> .....	0.14	1.0	2.0	4.2	4.2	2.5	2.7	2.1
Fe <sub>2</sub> O <sub>3</sub> .....	0.08	0.35	0.25	0.25	0.27	0.23	0.22	0.44
ZnO.....	.....	5.6	.....	10.9	10.9	7.8	3.6	.....
PbO.....	.....	.....	.....	.....	.....	.....	.....	1.0
MnO.....	0.02	0.02	0.01	0.01	0.01	0.01	0.03	0.03
CaO.....	8.7	0.66	0.29	0.63	0.56	0.79	2.6	0.42
MgO.....	0.17	4.3	0.06	0.21	0.25	3.4	2.6	0.08
Na <sub>2</sub> O.....	7.1	10.8	4.4	7.5	7.8	10.9	9.8	8.2
K <sub>2</sub> O.....	7.9	0.30	0.20	0.37	0.31	0.30	1.5	0.67
SiO <sub>2</sub> .....	75.9	73.0	80.5	64.7	64.7	67.3	68.6	75.9
B <sub>2</sub> O <sub>3</sub> .....	.....	3.6	11.8	10.6	10.6	6.2	8.1	10.8
P <sub>2</sub> O <sub>5</sub> .....	0.08	.....	.....	.....	.....	.....	.....	.....
So <sub>3</sub> .....	0.20	0.02	.....	.....	.....	.....	.....	.....
As <sub>2</sub> O <sub>3</sub> .....	Trace	0.02	0.70	0.14	0.19	Trace	0.18	0.36
Sb <sub>2</sub> O <sub>3</sub> .....	.....	0.60	.....	.....	.....	0.62	.....	.....
Total.....	100.29	100.27	100.21	99.81	99.79	100.05	99.93	100.00

Selenium and fluorine were not found, but lithium was detected spectroscopically by Paul W. Merrill in all the samples.

<sup>1</sup> P. H. Walker and F. W. Smither, Bur. Standards Tech. Papers No. 107 (1918).

## GENERAL SUMMARY OF TESTS ON CHEMICAL GLASSWARE

Table XLV gives a general summary of the resistance to various solutions and to mechanical and heat shock of chemical glassware tested by the Bureau of Standards. In this table the numerical exponents indicate the minor differences in resistance, the lowest number being the most resistant. The absence of an exponent indicates that the differences in resistance are too small to justify any differentiation between the wares graded in the same group. In the rating of resistance to caustic alkalis boiling tests only have been considered.

TABLE XLV

Ware	Resistance to						
	Water	Mineral acids	Carbonated alkalies	Caustic alkalies	Ammonia and ammonium salts	Heat shock	Mechanical shock
Kavalier.....	Poor	Good	Poor	Good <sup>2</sup>	Good <sup>2</sup>	Poor	Poor
M. E. G. Co.....	Good <sup>3</sup>	Good	Good <sup>1</sup>	Good <sup>1</sup>	Good	Poor	Poor
Pyrex.....	Good <sup>2</sup>	Good	Good <sup>3</sup>	Fair	Good	Good <sup>1</sup>	Good *
Jena.....	Good <sup>4</sup>	Good	Good <sup>2</sup>	Fair	Good	Good <sup>3</sup>	Fair
Nonsol.....	Good <sup>3</sup>	Good	Good <sup>1</sup>	Fair	Good	Good <sup>2</sup>	Fair
Fry.....	Good <sup>4</sup>	Good	Good <sup>2</sup>	Fair	Good	Poor	Good
Libbey.....	Good <sup>1</sup>	Good	Good <sup>3</sup>	Fair	Good	Good <sup>2</sup>	Good

\* Far superior to any of the other wares.

TABLE XLVI.—DATA ON THE STRENGTH OF AQUEOUS SOLUTIONS OF SOME COMMON ACIDS AND AMMONIA

Substance	sp. gr. 15.5° C.	Per Cent by Wt.	Normal	Molar
Ammonium hydroxide.....	0.90	28.5 NH <sub>3</sub>	15.1	15.1
Ammonium hydroxide.....	0.957	10.7 NH <sub>3</sub>	6	6
Hydrochloric acid.....	1.19	37.23 HCl	12	12
Hydrochloric acid.....	1.10	20.0 HCl	6	6
Nitric Acid.....	1.42	69.96 HNO <sub>3</sub>	15.8	15.8
Nitric acid.....	1.20	32.2 HNO <sub>3</sub>	6	6
Sulfuric acid.....	1.84	95.6 H <sub>2</sub> SO <sub>4</sub>	36	18
Sulfuric acid.....	1.19	26.0 H <sub>2</sub> SO <sub>4</sub>	6	3
Phosphoric acid.....	1.809*	93.67 H <sub>3</sub> PO <sub>4</sub>	5.2	1.7

\* At 17.5° C.

<sup>1</sup> P. H. Walker and F. W. Smither, Bur. Standards Tech. Papers No. 107 (1918).

TABLE XVII.—SOLUBILITY OF SOME GASES IN WATER, GRAMS PER LITER OF SOLVENT

When Partial Pressure of the Gas + Vapor Pressure of the Liquid = 760 mm.  
at the Respective Temperatures

T.	Oxygen*	Chlorine†	Carbon Dioxide‡	Hydrogen Sulfide§	Sulfur Dioxide
0	0.0695	.....	3.347	7.066	228.3
10	.0537	9.972	2.319	5.112	162.1
20	.0434	7.293	1.689	3.846	112.9
30	.0359	5.723	1.259	2.983	78.1
40	.0308	4.590	0.974	2.361	54.1
50	.0266	3.925	0.762	1.883	....
60	.0227	3.295	0.577	1.480	....
70	.0186	2.793	....	1.101	....
80	.0138	2.227	....	0.765	....
90	.0079	1.270	....	0.410	....
100	.0000	0.000	....	0.000	....

(From H. A. Fales, Inorganic Chemical Analysis, The Century Co., New York, 1925.)

\* L. W. Winkler, Ber. **22**, 1772 (1889).

† L. W. Winkler, Math. és Természettudományi Értesítő, **25**, 86 (1907).

‡ C. Bohr, Wied. Ann. **68**, 504 (1899).

§ L. W. Winkler, Math. és Természettudományi Értesítő, **25**, 86 (1907).

|| F. Schönfeld, Liebig's Ann. **95**, 1 (1855).

TABLE XLVIII.—CONVERSION TABLE OF UNITS OF LIQUID CAPACITY

Unit	Cubic Inches	Pounds of Water At 16.7°	U. S. Gallons	Imp. Gallons	Liters	Fluid Ounces
1 U. S. Gallon =	231.00	8.335	1.000	0.83	3.785	128.0
1 Imp. Gallon =	277.27	10.000	1.200	1.00	4.543	160.0
1 Liter =	61.03	2.200	0.264	0.22	1.0	33.8
1 Fluid Ounce =	1.80	0.065	0.008	0.006	0.029	1.0

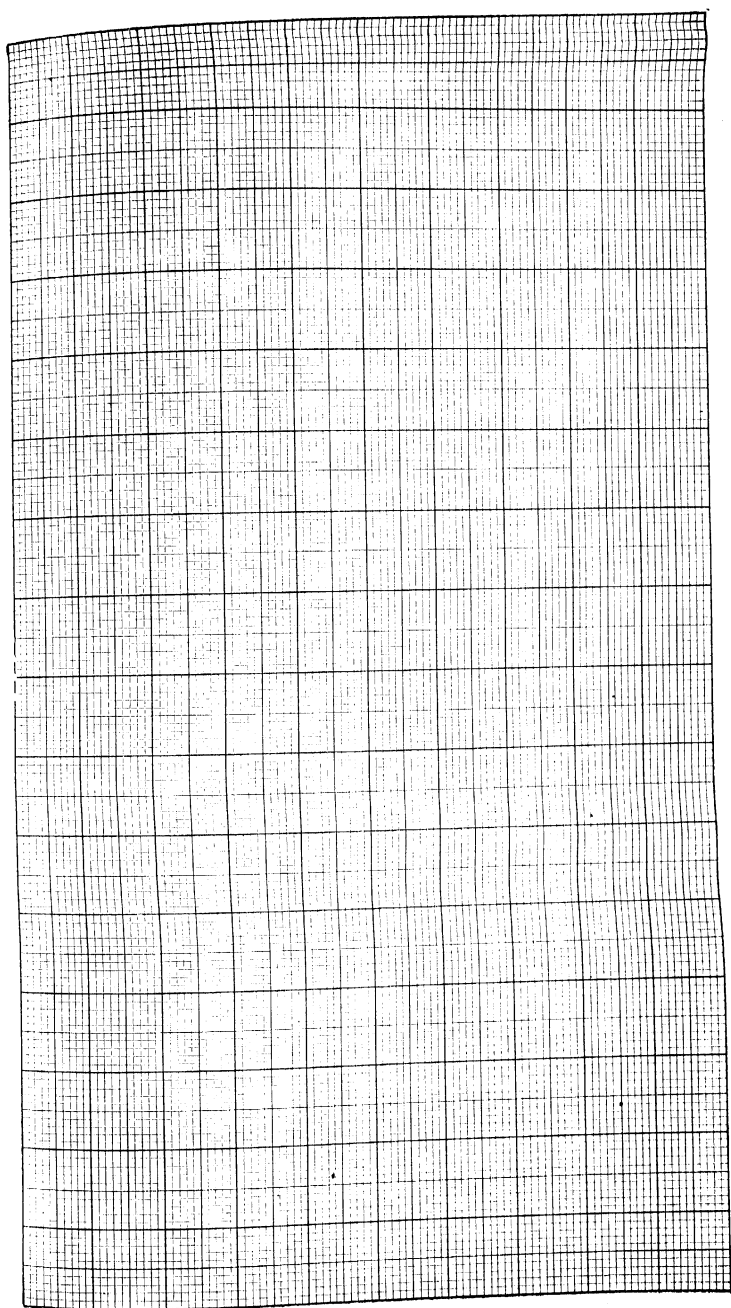
(From H. A. Fales, Inorganic Chemical Analysis, The Century Co., New York, 1925)

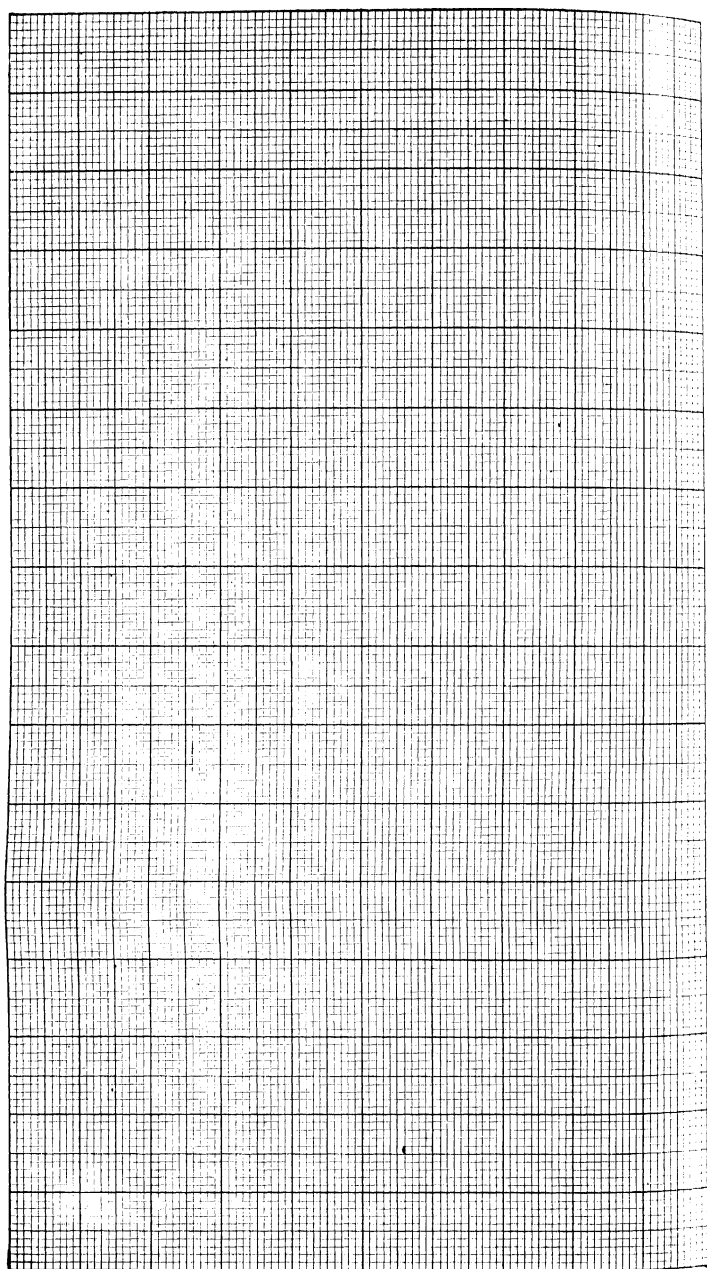


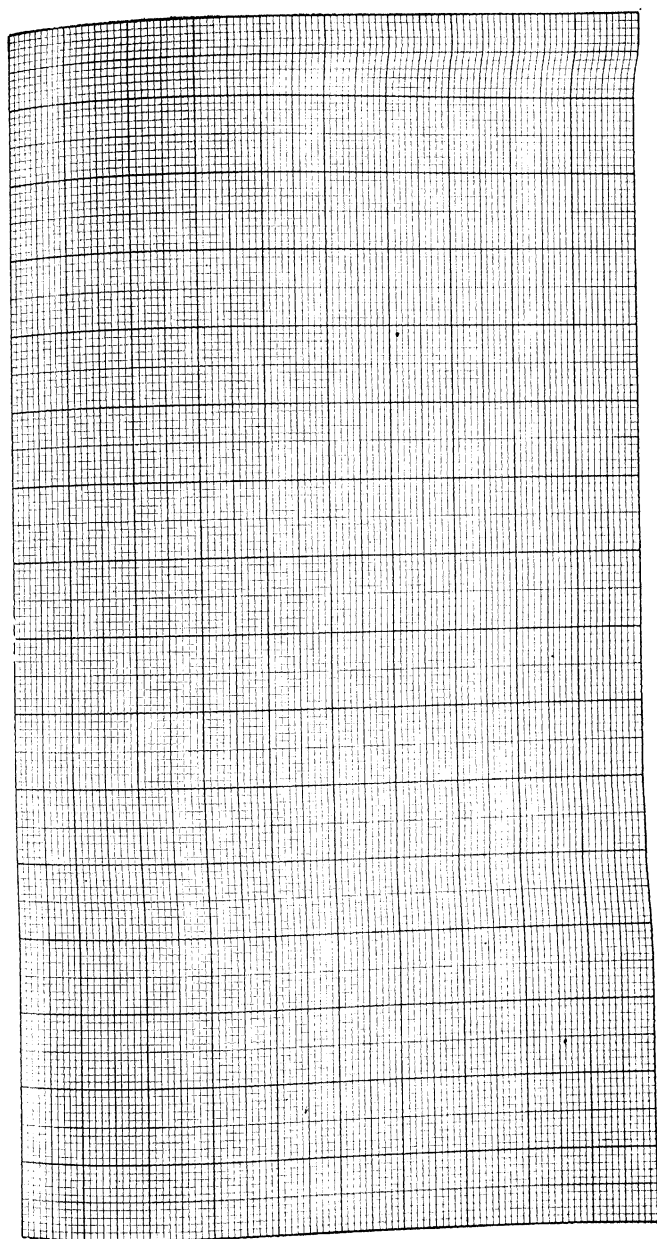
TABLE XLIX.—INTERNATIONAL TABLE OF ATOMIC WEIGHTS OF THE CHEMICAL ELEMENTS,\* 1925

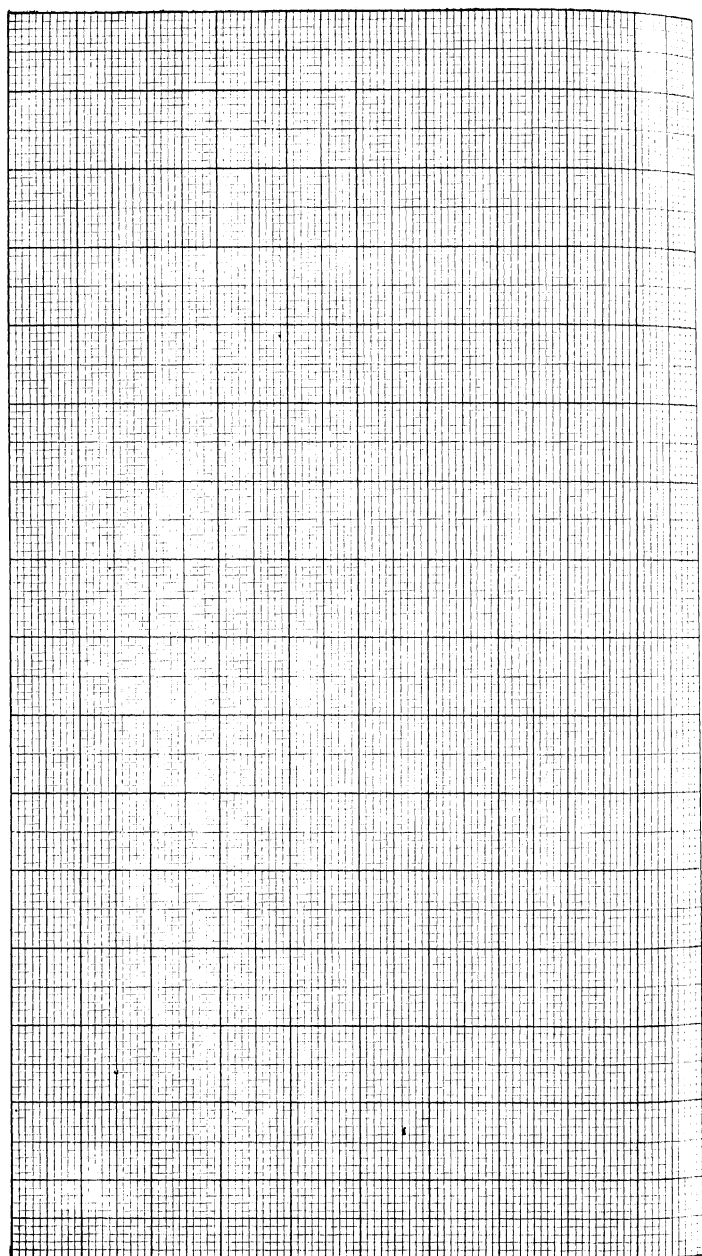
	Sym- bol	Atomic Number	Atomic Weight		Sym- bol	Atomic Number	Atomic Weight
Aluminum.....	Al	13	26.97	Molybdenum..	Mo	42	96.0
Antimony.....	Sb	51	121.77	Neodymium...	Nd	60	144.27
Argon.....	A	18	39.91	Neon.....	Ne	10	20.2
Arsenic.....	As	33	74.96	Nickel.....	Ni	28	58.69
Barium.....	Ba	56	137.37	Nitrogen.....	N	7	14.008
Beryllium.....	Be	4	9.02	Osmium.....	Os	76	190.8
Bismuth.....	Bi	83	209.00	Oxygen.....	O	8	16.000
Boron.....	B	5	10.82	Palladium.....	Pd	46	106.7
Bromine.....	Br	35	79.916	Phosphorus....	P	15	31.027
Cadmium.....	Cd	48	112.41	Platinum.....	Pt	78	195.23
Calcium.....	Ca	20	40.07	Potassium.....	K	19	39.096
Carbon.....	C	6	12.000	Praseodymium..	Pr	59	140.92
Cerium.....	Ce	58	140.25	Radium.....	Ra	88	225.95
Cesium.....	Cs	55	132.81	Radon.....	Rn	86	222.
Chlorine.....	Cl	17	35.457	Rhodium.....	Rh	45	102.91
Chromium.....	Cr	24	52.01	Rubidium.....	Rb	37	85.44
Cobalt.....	Co	27	58.94	Ruthenium.....	Ru	44	101.7
Columbium.....	Cb	41	93.1	Samarium.....	Sm	62	150.43
Copper.....	Cu	29	63.57	Scandium.....	Sc	21	45.10
Dysprosium.....	Dy	66	162.52	Selenium.....	Se	34	79.2
Erbium.....	Er	68	167.7	Silicon.....	Si	14	28.06
Europium.....	Eu	63	152.0	Silver.....	Ag	47	107.880
Fluorine.....	F	9	19.00	Sodium.....	Na	11	22.997
Gadolinium.....	Gd	64	157.26	Strontium.....	Sr	38	87.63
Gallium.....	Ga	31	69.72	Sulfur.....	S	16	32.064
Germanium.....	Ge	32	72.60	Tantalum.....	Ta	73	181.5
Gold.....	Au	79	197.2	Tellurium.....	Te	52	127.5
Helium.....	He	2	4.00	Terbium.....	Tb	65	159.2
Holmium.....	Ho	67	163.4	Thallium.....	Tl	81	204.39
Hydrogen.....	H	1	1.008	Thorium.....	Th	90	232.15
Indium.....	In	49	114.8	Thulium.....	Tm	69	169.4
Iodine.....	I	53	126.932	Tin.....	Sn	50	118.70
Iridium.....	Ir	77	193.1	Titanium.....	Ti	22	48.1
Iron.....	Fe	26	55.84	Tungsten.....	W	74	184.0
Krypton.....	Kr	36	82.9	Uranium.....	U	92	238.17
Lanthanum.....	La	57	138.90	Vanadium.....	V	23	50.96
Lead.....	Pb	82	207.20	Xenon.....	Xe	54	130.2
Lithium.....	Li	3	6.940	Ytterbium.....	Yb	70	173.6
Lutecium.....	Lu	71	175.0	Yttrium.....	Y	39	88.9
Magnesium.....	Mg	12	24.32	Zinc.....	Zn	30	65.38
Manganese.....	Mn	25	54.93	Zirconium.....	Zr	40	91.
Mercury.....	Hg	80	200.61				

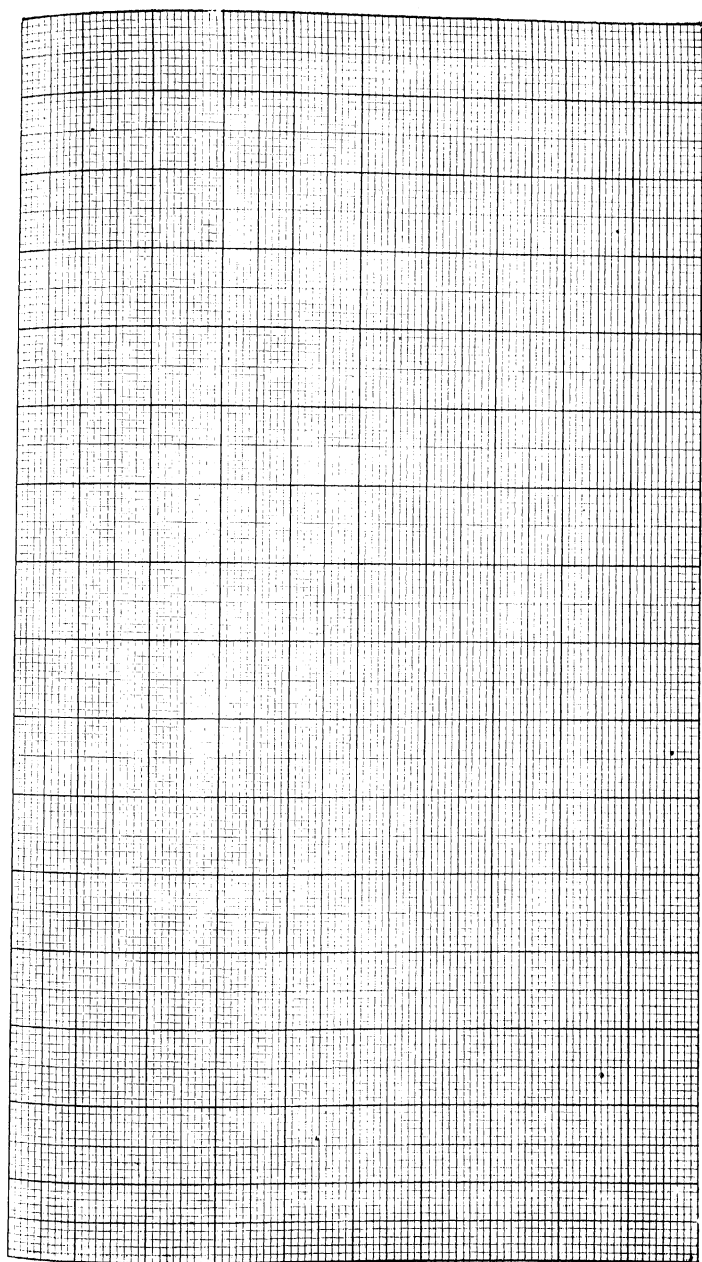
\* *Note by the Author.*—The table of Atomic Weights as issued by the International Committee lists the elements alphabetically according to their symbol. In the above table the elements are arranged alphabetically according to their names. The table does not include element No. 72, Hafnium or Celium, atomic weight 180.8. For information regarding this atomic weight, and also for more recent information regarding other atomic weights, see "Annual Report of the Committee on Atomic Weights," G. P. Baxter, J. Am. Chem. Soc. **47**, 601 (1925); *ibid.* **48**, 541 (1926); *ibid.* **49**, 583 (1927); *ibid.* **50**, 603 (1928).

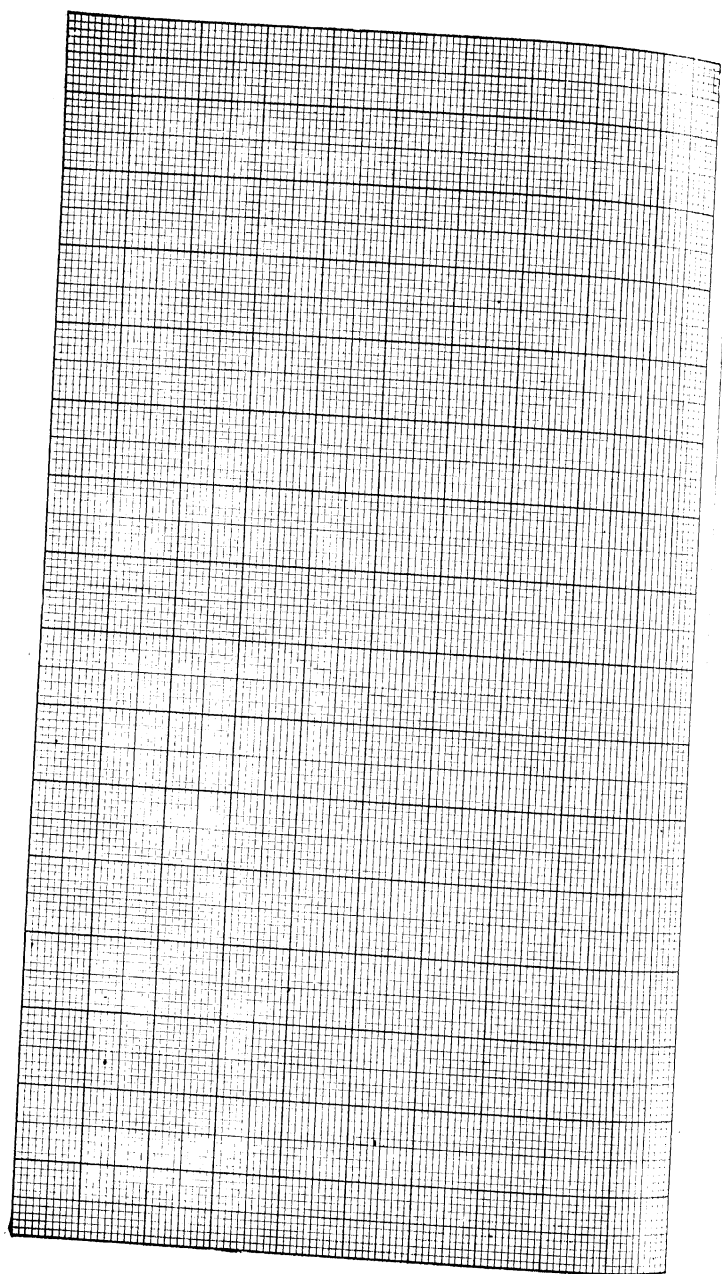


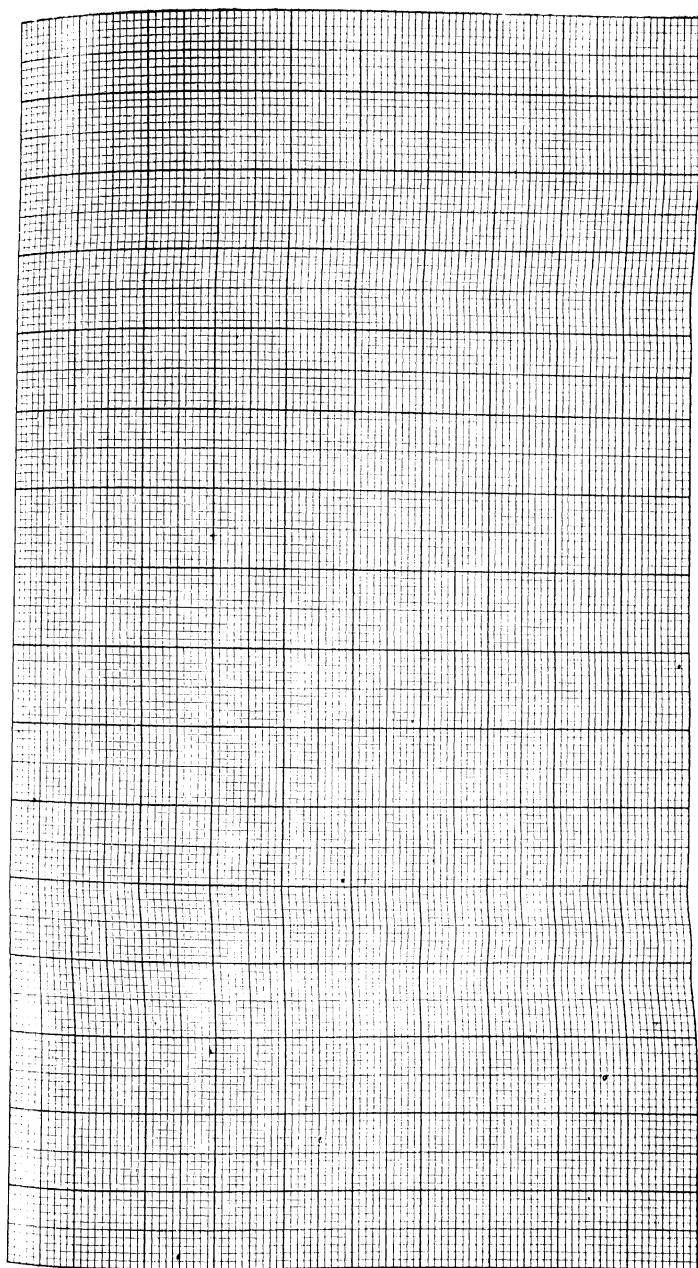




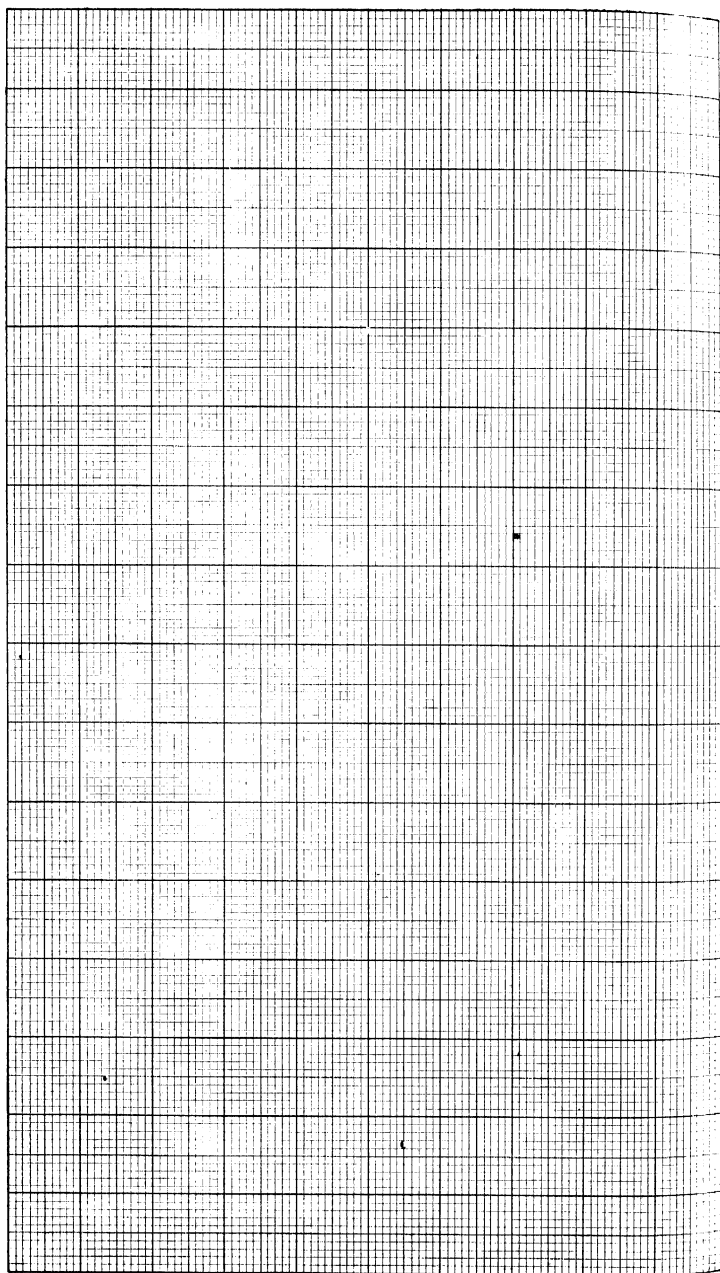












## LOGARITHMS

## LOGARITHMS OF NUMBERS

## LOGARITHMS OF NUMBERS

Natural num- bers.											PROPORTIONAL PARTS									
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4	8	12	17	21	25	29	33	37	
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34	
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31	
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29	
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27	
15	1761	1790	1818	1847	1875	1903	1931	1958	1987	2014	3	6	8	11	14	17	20	22	25	
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24	
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22	
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21	
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20	
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19	
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18	
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17	
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17	
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16	
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15	
26	4150	4166	4183	4200	4217	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15	
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14	
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14	
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13	
30	4771	4786	4800	4815	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13	
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12	
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12	
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12	
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11	
35	5441	5453	5465	5478	5490	5502	5514	5527	5538	5551	1	2	4	5	6	7	9	10	11	
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11	
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10	
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10	
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	5	7	8	9	10	
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10	
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9	
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9	
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9	
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9	
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9	
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	8	9	
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	6	7	8	9	
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	5	6	7	8	9	
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	5	6	7	8	9	
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	4	5	6	7	8	9	
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	4	5	6	7	8	9	
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	3	4	5	6	7	8	9	
53	7243	7251	7259	7267	7275	7282	7290	7300	7308	7316	1	2	3	4	5	6	7	8	9	
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	3	4	5	6	7	8	9	

LOGARITHMS OF NUMBERS (Continued)

Natural numbers.											PROPORTIONAL PARTS.									
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7	
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7	
57	7559	7566	7574	7582	7589	7597	7604	7612	7616	7622	1	2	2	3	4	5	5	6	7	
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7	
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7	
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6	
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6	
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6	
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6	
64	8062	8069	8075	8082	8088	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6	
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	4	5	6	
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	4	5	6	
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8318	1	1	2	3	3	4	4	5	6	
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6	
69	8388	8395	8401	8407	8414	8420	8426	8432	8438	8445	1	1	2	2	3	4	4	5	6	
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6	
71	8513	8519	8525	8531	8537	8543	8548	8555	8561	8567	1	1	2	2	3	4	4	5	5	
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5	
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5	
74	8692	8698	8704	8710	8716	8722	8727	8733	8738	8745	1	1	2	2	3	4	4	5	5	
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	4	5	
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	4	5	
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5	
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5	
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9026	1	1	2	2	3	3	4	4	5	
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5	
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5	
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5	
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5	
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5	
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5	
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5	
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	1	1	2	2	3	3	4	4	5	
88	9445	9450	9455	9460	9465	9470	9475	9480	9484	9489	1	1	2	2	3	3	4	4	5	
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	1	1	2	2	3	3	4	4	5	
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	1	1	2	2	3	3	4	4	5	
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	1	1	2	2	3	3	4	4	5	
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	1	1	2	2	3	3	4	4	5	
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	1	1	2	2	3	3	4	4	5	
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	1	1	2	2	3	3	4	4	5	
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	1	1	2	2	3	3	4	4	5	
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	1	1	2	2	3	3	4	4	5	
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	1	1	2	2	3	3	4	4	5	
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	1	1	2	2	3	3	4	4	5	
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	1	1	2	2	3	3	4	4	5	

## ANTILOGARITHMS

Logarithms.											PROPORTIONAL PARTS.									
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	
.00	1000	1002	1005	1007	1009	1012	1014	1016	1019	1021	0	0	1	1	1	1	2	2	2	
.01	1023	1026	1028	1030	1033	1035	1038	1040	1042	1045	0	0	1	1	1	1	2	2	2	
.02	1047	1050	1052	1054	1057	1059	1062	1064	1067	1069	0	0	1	1	1	1	2	2	2	
.03	1072	1074	1076	1079	1081	1084	1086	1089	1091	1094	0	0	1	1	1	1	2	2	2	
.04	1096	1099	1102	1104	1107	1109	1112	1114	1117	1119	0	1	1	1	1	2	2	2		
.05	1122	1125	1127	1130	1132	1135	1138	1140	1143	1146	0	1	1	1	1	2	2	2		
.06	1148	1151	1153	1156	1159	1161	1164	1167	1169	1172	0	1	1	1	1	2	2	2		
.07	1175	1178	1180	1183	1186	1189	1191	1194	1197	1199	0	1	1	1	1	2	2	2		
.08	1202	1205	1208	1211	1213	1216	1219	1222	1225	1227	0	1	1	1	1	2	2	2		
.09	1230	1233	1236	1239	1242	1245	1247	1250	1253	1256	0	1	1	1	1	2	2	2	3	
.10	1259	1262	1265	1268	1271	1274	1276	1279	1282	1285	0	1	1	1	1	2	2	2	3	
.11	1288	1291	1294	1297	1300	1303	1306	1309	1312	1315	0	1	1	1	1	2	2	2	3	
.12	1318	1321	1324	1327	1330	1334	1337	1340	1343	1346	0	1	1	1	1	2	2	2	3	
.13	1349	1352	1355	1358	1361	1365	1368	1371	1374	1377	0	1	1	1	1	2	2	2	3	
.14	1380	1384	1387	1390	1393	1396	1400	1403	1406	1409	0	1	1	1	1	2	2	2	3	
.15	1413	1416	1419	1422	1426	1429	1432	1435	1439	1442	0	1	1	1	1	2	2	2	3	
.16	1445	1449	1452	1455	1459	1462	1466	1469	1472	1476	0	1	1	1	1	2	2	2	3	
.17	1479	1483	1486	1489	1493	1496	1500	1503	1507	1510	0	1	1	1	1	2	2	2	3	
.18	1514	1517	1521	1524	1528	1531	1535	1538	1542	1545	0	1	1	1	1	2	2	2	3	
.19	1549	1552	1556	1560	1563	1567	1570	1574	1578	1581	0	1	1	1	1	2	2	2	3	
.20	1585	1589	1592	1596	1600	1603	1607	1611	1614	1618	0	1	1	1	1	2	2	2	3	
.21	1622	1626	1629	1633	1637	1641	1644	1648	1652	1656	0	1	1	1	1	2	2	2	3	
.22	1660	1663	1667	1671	1675	1679	1683	1687	1690	1694	0	1	1	1	1	2	2	2	3	
.23	1698	1702	1706	1710	1714	1718	1722	1726	1730	1734	0	1	1	1	1	2	2	2	3	
.24	1738	1742	1746	1750	1754	1758	1762	1766	1770	1774	0	1	1	1	1	2	2	2	3	
.25	1778	1782	1786	1791	1795	1799	1803	1807	1811	1816	0	1	1	1	1	2	2	2	3	
.26	1820	1824	1828	1832	1837	1841	1845	1849	1854	1858	0	1	1	1	1	2	2	2	3	
.27	1862	1866	1871	1875	1879	1884	1888	1892	1897	1901	0	1	1	1	1	2	2	2	3	
.28	1905	1910	1914	1919	1923	1928	1932	1936	1941	1945	0	1	1	1	1	2	2	2	3	
.29	1950	1954	1959	1963	1968	1972	1977	1982	1986	1991	0	1	1	1	1	2	2	2	3	
.30	1995	2000	2004	2009	2014	2018	2023	2028	2032	2037	0	1	1	1	1	2	2	2	3	
.31	2042	2046	2051	2056	2061	2065	2070	2075	2080	2084	0	1	1	1	1	2	2	2	3	
.32	2089	2094	2099	2104	2109	2113	2118	2123	2128	2133	0	1	1	1	1	2	2	2	3	
.33	2138	2143	2148	2153	2158	2163	2168	2173	2178	2183	0	1	1	1	1	2	2	2	3	
.34	2188	2193	2198	2203	2208	2213	2218	2223	2228	2234	1	1	1	1	2	2	2	2	3	
.35	2239	2244	2249	2254	2259	2265	2270	2275	2280	2286	1	1	1	1	2	2	2	2	3	
.36	2291	2296	2301	2307	2312	2317	2323	2328	2333	2339	1	1	1	1	2	2	2	2	3	
.37	2344	2350	2355	2360	2366	2371	2377	2382	2388	2393	1	1	1	1	2	2	2	2	3	
.38	2399	2404	2410	2415	2421	2427	2432	2438	2443	2449	1	1	1	1	2	2	2	2	3	
.39	2455	2460	2466	2472	2477	2483	2489	2495	2500	2506	1	1	1	1	2	2	2	2	3	
.40	2512	2518	2523	2529	2535	2541	2547	2553	2559	2564	1	1	1	1	2	2	2	2	3	
.41	2570	2576	2582	2588	2594	2600	2606	2612	2618	2624	1	1	1	1	2	2	2	2	3	
.42	2630	2636	2642	2649	2655	2661	2667	2673	2679	2685	1	1	1	1	2	2	2	2	3	
.43	2692	2698	2704	2710	2716	2723	2729	2735	2742	2748	1	1	1	1	2	2	2	2	3	
.44	2754	2761	2767	2773	2780	2786	2793	2799	2805	2812	1	1	1	1	2	2	2	2	3	
.45	2818	2825	2831	2838	2844	2851	2858	2864	2871	2877	1	1	1	1	2	2	2	2	3	
.46	2884	2891	2897	2904	2911	2917	2924	2931	2938	2944	1	1	1	1	2	2	2	2	3	
.47	2951	2958	2965	2972	2979	2985	2992	2999	3006	3013	1	1	1	1	2	2	2	2	3	
.48	3020	3027	3034	3041	3048	3055	3062	3069	3076	3083	1	1	1	1	2	2	2	2	3	
.49	3090	3097	3105	3112	3119	3126	3133	3141	3148	3155	1	1	1	1	2	2	2	2	3	

## ANTILOGARITHMS (Continued)

Logarithms.											PROPORTIONAL PARTS.									
	0	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	
.50	3162	3170	3177	3184	3192	3199	3206	3214	3221	3228	1	1	2	3	4	5	6	7		
.51	3236	3243	3251	3258	3266	3273	3281	3289	3296	3304	1	2	2	3	4	5	6	7		
.52	3311	3319	3327	3334	3342	3350	3357	3365	3373	3381	1	2	2	3	4	5	6	7		
.53	3388	3396	3404	3412	3420	3428	3436	3443	3451	3459	1	2	2	3	4	5	6	7		
.54	3467	3475	3483	3491	3499	3508	3516	3524	3532	3540	1	2	2	3	4	5	6	7		
.55	3548	3556	3565	3573	3581	3589	3597	3606	3614	3622	1	2	2	3	4	5	6	7		
.56	3631	3639	3648	3656	3664	3673	3681	3690	3698	3707	1	2	3	3	4	5	6	7		
.57	3715	3724	3733	3741	3750	3758	3767	3776	3784	3793	1	2	3	3	4	5	6	7		
.58	3802	3811	3819	3828	3837	3846	3855	3864	3873	3882	1	2	3	4	4	5	6	7		
.59	3890	3899	3908	3917	3926	3935	3945	3954	3963	3972	1	2	3	4	5	6	7	8		
.60	3981	3990	3999	4009	4018	4027	4036	4046	4055	4064	1	2	3	4	5	6	7	8		
.61	4074	4083	4093	4102	4111	4121	4130	4140	4150	4159	1	2	3	4	5	6	7	8		
.62	4169	4178	4188	4198	4207	4217	4227	4236	4246	4256	1	2	3	4	5	6	7	8		
.63	4266	4276	4285	4295	4305	4315	4325	4335	4345	4355	1	2	3	4	5	6	7	8		
.64	4365	4375	4385	4395	4406	4416	4426	4436	4446	4457	1	2	3	4	5	6	7	8		
.65	4467	4477	4487	4498	4508	4519	4529	4539	4550	4560	1	2	3	4	5	6	7	8		
.66	4571	4581	4592	4603	4613	4624	4634	4645	4656	4667	1	2	3	4	5	6	7	9	10	
.67	4677	4688	4699	4710	4721	4732	4742	4753	4764	4775	1	2	3	4	5	7	8	9	10	
.68	4786	4797	4808	4819	4831	4842	4853	4864	4875	4887	1	2	3	4	6	7	8	9	10	
.69	4898	4909	4920	4932	4943	4955	4966	4977	4988	5000	1	2	3	5	6	7	8	9	10	
.70	5012	5023	5035	5047	5058	5070	5082	5093	5105	5117	1	2	4	5	6	7	8	9	11	
.71	5129	5140	5152	5164	5176	5188	5200	5212	5224	5236	1	2	4	5	6	7	8	10	11	
.72	5248	5260	5272	5284	5297	5309	5321	5333	5346	5358	1	2	4	5	6	7	9	10	11	
.73	5370	5383	5395	5408	5420	5433	5445	5458	5470	5483	1	3	4	5	6	8	9	10	11	
.74	5495	5508	5521	5534	5546	5559	5572	5585	5598	5610	1	3	4	5	6	8	9	10	12	
.75	5623	5636	5649	5662	5675	5689	5702	5715	5728	5741	1	3	4	5	7	8	9	10	12	
.76	5754	5768	5781	5794	5808	5821	5834	5848	5861	5875	1	3	4	5	7	8	9	11	12	
.77	5888	5902	5916	5929	5943	5957	5970	5984	5998	6012	1	3	4	5	7	8	10	11	12	
.78	6026	6039	6053	6067	6081	6095	6109	6124	6138	6152	1	3	4	6	7	8	10	11	13	
.79	6166	6180	6194	6209	6223	6237	6252	6266	6281	6295	1	3	4	6	7	9	10	11	13	
.80	6310	6324	6339	6353	6368	6383	6397	6412	6427	6442	1	3	4	6	7	9	10	12	13	
.81	6457	6471	6486	6501	6516	6531	6546	6561	6577	6592	2	3	5	6	8	9	11	12	14	
.82	6607	6622	6637	6653	6668	6683	6699	6714	6730	6745	2	3	5	6	8	9	11	12	14	
.83	6761	6776	6792	6808	6823	6839	6855	6871	6887	6902	2	3	5	6	8	9	11	13	14	
.84	6918	6934	6950	6966	6982	6998	7015	7031	7047	7063	2	3	5	6	8	10	11	13	15	
.85	7079	7096	7112	7129	7145	7161	7178	7194	7211	7228	2	3	5	7	8	10	12	14	15	
.86	7244	7261	7278	7295	7311	7328	7345	7362	7379	7396	2	3	5	7	8	10	12	13	15	
.87	7413	7430	7447	7464	7482	7499	7516	7534	7551	7568	2	3	5	7	9	10	12	14	16	
.88	7586	7603	7621	7638	7656	7674	7691	7709	7727	7745	2	4	5	7	9	11	12	14	16	
.89	7762	7780	7798	7816	7834	7852	7870	7889	7907	7925	2	4	5	7	9	11	13	14	16	
.90	7943	7962	7980	7998	8017	8035	8054	8072	8091	8110	2	4	6	7	9	11	13	15	17	
.91	8128	8147	8166	8185	8204	8222	8241	8260	8279	8299	2	4	6	8	9	11	13	15	17	
.92	8318	8337	8356	8375	8395	8414	8433	8453	8472	8492	2	4	6	8	10	12	14	15	17	
.93	8511	8531	8551	8570	8590	8610	8630	8650	8670	8690	2	4	6	8	10	12	14	16	18	
.94	8710	8730	8750	8770	8790	8810	8831	8851	8872	8892	2	4	6	8	10	12	14	16	18	
.95	8913	8933	8954	8974	8995	9016	9036	9057	9078	9099	2	4	6	8	10	12	14	15	17	
.96	9120	9141	9162	9183	9204	9226	9247	9268	9289	9311	2	4	6	8	11	13	15	17	19	
.97	9333	9354	9376	9397	9419	9441	9462	9484	9506	9528	2	4	7	9	11	13	15	17	20	
.98	9550	9572	9594	9616	9638	9661	9683	9705	9727	9750	2	4	7	9	11	13	16	18	20	
.99	9772	9795	9817	9840	9863	9886	9908	9931	9954	9977	2	5	7	9	11	14	16	18	20	



## AUTHOR INDEX

*(References to the Bibliography are in Italics)*

### A

ABDERHALDEN, E., 705  
 ABELOUS, J. E., 538  
 ABRAMS, D. A., 664  
 ACÉL, D., 646, 656  
 ACKERSON, C. W., 674  
 ACREE, S. F., 541  
 ADAMS, E. T., 264, 266, 627  
 ADDIS, T., 597, 689  
 ADLER, A., 577  
 ADLER, L., 544  
 ADOLPH, W. H., 709  
 ADOVA, A. N., 666, 703  
 AGULHON, H., 135, 540, 556  
 ALBRECHT, P. G., 587, 592, 711  
 ALESSANDRIA, 668  
 ALEXANDER, J., 93, 98  
 ALLEN, 252  
 ALLEN, A. H., 556, 622  
 ALLEN, D. M., 474, 567  
 ALLEN, E. R., 548, 646  
 ALLEN, W. S., 126, 551  
 ALLERS, R., 538  
 ALLIN, K. D., 662, 708  
 ALMY, L. H., 610  
 ALVAREZ, E. P., 563, 641, 642, 652, 653  
 ALVAY, F. J., 605  
 AMBERG, S., 609  
 AMBÜHL, G., 685  
 AMOSS, H. L., 605  
 ANDERSON, R. J., 590, 710  
 ANDERSON, V. G., 624  
 ANDREW, R. I., 252, 254, 625  
 ANDREWS, L. W., 642  
 APPLEBAUM, A. I., 166, 569  
 APPLETON, V. B., 603  
 ARDAGH, E. G. R., 355, 676  
 ARGENSON, G., 540  
 ARMANI, G., 654

ARMIT, H. W., 637  
 ARMSTRONG, H. E., 545  
 ARNAUD, F. W. F., 556  
 ARNOLD, J. O., 562, 695  
 ARNY, H. V., 3, 251, 548, 646, 656, 671, 680,  
     684, 685, 710, 716  
 ARTUS, 546  
 ASCHNER, P. W., 688  
 ASTRUC, A., 631  
 ATACK, F. W., 119, 120, 173, 542, 571, 637  
 ATKINS, W. R. G., 552, 676, 683  
 ATKINSON, H. V., 555  
 ATTERBERG, A., 551  
 AUBRY, P., 133, 554  
 AUCHY, G., 561, 629  
 AUERBACH, F., 657  
 AUGER, V., 571  
 AUSTIN, A., 582  
 AUSTIN, J. H., 493, 606, 607, 660  
 AUTENRIETH, W., 530, 547, 554, 565, 575,  
     585, 589, 596, 600, 609, 610, 617, 621, 623,  
     633, 645, 655, 677, 686, 687, 709  
 AVERY, D., 624

### B

BABBITT, H. C., 629, 696  
 BACH, A., 582, 658  
 BACH, H., 431, 666  
 BADER, J. P., 579  
 BADO, A. A., 704  
 BAEHR, G., 687  
 BAERWALD, 334  
 BAGG, H. J., 502, 711  
 BAILEY, 216, 217  
 BAILEY, C. V., 472, 688, 712  
 BAILEY, D. F. H., 594, 699  
 BAILEY, K. C., 594, 699  
 BAKER, F. E., 664  
 BAKER, W., 586, 590, 660, 710



- BALDWIN, F. O., 543  
 BANCROFT, W. D., 97, 99, 399  
 BANKS, H. W., 528  
 BARBET, 651  
 BARBET, E., 579  
 BARBONI, J., 654  
 BARBOUR, H. G., 606  
 BARDACH, F., 631  
 BARKER, P., 369, 370, 371, 669  
 BARKUS, O., 600, 620, 675  
 BARLEY, C. V., 691  
 BARNEBEY, O. L., 701  
 BARR, D. P., 691  
 BARRON, E. G., 552  
 BARSCHALL, E. B. H., 584, 588  
 BARTH, M., 581  
 BARTHELEMY, H., 537  
 BARTOW, E., 318, 643  
 BARTRAM, G. H., 640  
 BASKERVILLE, C., 700  
 BASSETT, H., JR., 616  
 BAUBIGNY, H., 557  
 BAUDOUIN, A., 576, 577  
 BAUMAN, L., 711  
 BAUMANN, E., 611  
 BAUMANN, E. J., 567, 587, 591, 673, 688  
 BAUMANN, L., 585, 586, 590  
 BAUR, E., 585  
 BAXTER, G. P., 722  
 BAY, I., 642  
 BAYLEY, T., 573, 581  
 BAYLIS, J. R., 704  
 BEAM, W., 125, 550, 659  
 BEANS, 96  
 BECHHOLD, 92  
 BECKER, W., 630  
 BEELER, C., 592  
 BEER, 2, 52, 60, 70, 72, 74, 75, 77, 80, 81, 108  
 BEHRE, J. A., 466, 538, 587, 592, 708  
 BELL, J. C., 614  
 BELL, R., 591, 661  
 BELL, R. D., 264, 346, 513, 514, 548, 671, 684  
 BÉNARD, H., 576, 577  
 BÉNAZET, P., 668  
 BENEDICT, E. M., 588, 593  
 BENEDICT, F. G., 584, 588  
 BENEDICT, S. R., 11, 26, 55, 57, 353, 448, 458, 462, 466, 471, 472, 478, 479, 481, 482, 500, 505, 519-22, 525, 526, 538, 549, 576, 585, 587, 588, 592, 593, 660, 667, 668, 674, 687, 689, 690, 692, 693, 709, 711, 712, 713  
 BENEKER, J. C., 562  
 BENNETT, C. T., 623  
 BENNETT, M. A., 608  
 BERCELLER, L., 603  
 BERGEIM, O., 348, 353, 451, 457, 478, 480, 511, 545, 550, 552, 567, 588, 593, 601, 611, 627, 663, 668, 675, 678, 693, 708, 713  
 BERGER, H., 657  
 BERGERON, 581  
 BERGLUND, H., 690  
 BERKEFELD, 122  
 BERL, E., 594  
 BERMAN, L., 618  
 BERNHARD, A., 559, 566, 687  
 BERNOUILLI, A. L., 550, 579, 620, 626, 659  
 BERNSTEIN, A., 574  
 BERNSTROP, J. C., 622  
 BERNVALLI, A., 576  
 BERTHELOT, 406, 538  
 BERTRAND, 368, 683  
 BERTRAND, G., 135, 556, 631  
 BETTEL, W., 283, 602, 634  
 BEVON, 444  
 BEYTHIEN, A., 542  
 BIERMAN, H. R., 621  
 BIGELOW, W. D., 680  
 BILHAM, P., 584  
 BINET, L., 586, 590  
 BING, H. I., 599  
 BIRCKNER, V., 99, 397, 399, 718  
 BIRD, L. A., 699  
 BISCHOF, G., 252, 622  
 BISHOP, G. H., 662  
 BLACK, A., 673  
 BLACK, O. F., 551  
 BLACKFAN, K. D., 567  
 BLAIR, J. A., 640  
 BLAKE, S. A., 682  
 BLANC, G., 655  
 BLATHERWICK, N. R., 586, 668  
 BLAU, N. F., 592  
 BLISH, M. J., 674  
 BLOOH, M., 677  
 BLOOR, W. R., 474, 478, 566, 567, 671, 672, 678  
 BLOUNT, B., 614  
 BLOWSKI, 217  
 BLUM, 110  
 BLUNT, K., 588, 593

- BLUNT, T. P., 581, 638  
 BLUTH, F., 537  
 BOBIER, M., 575  
 BOCK, J., 621  
 BOCK, J. C., 11, 26, 55, 448, 500, 505, 576,  
 660, 663, 692, 707  
 BODANSKY, A., 707  
 BODANSKY, M., 99, 397, 399, 583, 691, 718  
 BODMER, R., 582  
 BOGERT, L. J., 590, 627, 710  
 BOGITCH, F., 562  
 BOHN, 216  
 BOHN, R. M., 612  
 BOHR, C., 721  
 BOISMENU, E., 407, 538  
 BOIVIN, A., 713  
 BOLAS, T., 639  
 BONIS, A., 577  
 BONIS, M. A., 541  
 BONNET, F., JR., 418, 419, 541  
 BOOHER, L. E., 606, 607  
 BOOTH, H. S., 633  
 BOOTHBY, W. M., 592  
 BORDAS, F., 601  
 BORNAND, M., 655  
 BORNTRÄGER, A., 615  
 BORRIEN, V., 686  
 BORSOOK, H., 703, 706  
 BOSWORTH, A. W., 590, 710  
 BÖTTGER, 649  
 BÖTTGER, R., 613, 639  
 BOTTOMLEY, J., 573, 579  
 BOUGHTON, W. A., 635  
 BOUIS, M. A., 540  
 BOURCET, P., 612  
 BOURNE, W., 606  
 BOUSSU, R., 558  
 BOUTOT, L., 691  
 BOWERS, W. G., 577  
 BOYD, J. I., 675  
 BOYNTON, H. C., 562  
 BOYNTON, H. K., 562  
 BRADFIELD, R., 216  
 BRADLEY, H. C., 582, 674  
 BRAKES, J., 701  
 BRALEY, S. A., 571  
 BRAMLEY, A., 618, 699  
 BRANOWER, J., 688  
 BRATKOWSKI, W., 574  
 BRAUN, A. D., 634  
 BRAUN, C. D., 472  
 BREAZEALE, 363  
 BRETEAU, P., 256, 624  
 BREYER, 99, 397  
 BRIGGS, A. P., 264, 346-8, 605, 627, 672, 673  
 BRITTON, J. B., 560, 572  
 BRODIGAN, C. B., 602  
 BROTHERHOOD, J. S., 576  
 BROWN, A. L., 619  
 BROWN, B. E., 268, 339-41, 669  
 BROWN, F. J., 562  
 BROWN, H., 463, 712, 713  
 BROWN, J. H., 213  
 BROWN, J. W., 630  
 BRUNCK, O., 681  
 BRÜNNER, A., 628  
 BRUTTINI, A., 706  
 BUCHANAN, G. S., 700  
 BÜCHNER, 299, 457, 458, 525  
 BUCKMAN, T. E., 585  
 BUDDEN, E. R., 253, 582, 615, 622, 633, 700  
 BUELL, M. V., 673  
 BUISSON, A., 546  
 BULMER, F. M. R., 466, 713  
 BURGESS, W. T., 665  
 BÜRKER, K., 578  
 BURTON, E. F., 100, 399  
 BUTTERFIELD, 490  
  
 C  
 CAIN, J. R., 100, 101, 156, 218, 219, 222,  
 224-26, 229-33, 333, 574, 616  
 CAJORI, F. A., 675, 691, 692  
 CALDWELL, M. L., 606  
 CALVERT, E. G. B., 690  
 CAMERON, A. T., 612  
 CAMERON, C. A., 579  
 CAMERON, F. K., 359-64, 677  
 CAMPBELL, E. D., 10, 21, 22, 110, 403, 575  
 CAMPBELL, W. R., 586, 587, 591, 597, 598  
 CAMPO Y CERDAN, A. DEL, 396, 717  
 CANNON, W. B., 539  
 CARLES, P., 253  
 CARLETON, P. W., 30  
 CARNELLEY, T., 581, 613  
 CARNOT, A., 601  
 CARON, H., 643-5  
 CARPENTIER, G., 583  
 CARPIAUX, E., 143, 558  
 CARR, R. H., 684  
 CARRÂCIDO, J. R., 633  
 CARRÉ, L., 666

- CARTER, R. H., 647  
 CASSAL, C. E., 135, 556  
 CASSAL, N. C., 680  
 CASSEL, C., 685  
 CASSEL, H. R., 191, 192, 601  
 CATFORD, J. P., 599  
 CATHCART, E. P., 590  
 CAVALIER, 546  
 CAYVAN, I. L., 337, 682  
 CAZENEUVE, M., 166, 255, 256, 568  
 CAZENEUVE, P., 641  
 CHACE, F. M., 414, 570  
 CHALLINOR, R. W., 570, 637  
 CHAMBERS, W. H., 606  
 CHAMOT, E. M., 313, 643, 644  
 CHANEY, M. S., 588, 593  
 CHANUTIN, A., 594  
 CHAPIN, R. M., 540, 594, 667  
 CHAPMAN, 472  
 CHAPMAN, A. C., 588, 594, 598  
 CHAPMAN, E. T., 545, 638  
 CHARNASS, D., 617  
 CHEATHAM, P. N., 586, 590  
 CHEEVER, B. W., 629  
 CHEN, K. K., 674  
 CHÉNEVEAU, C., 558  
 CHERAMY, P., 664  
 CHERTKOV, I., 591  
 CHESNEAU, 346  
 CHESNUT, R. K., 589  
 CHOUGHAK, D., 130, 546, 552, 670  
 CHOUKE, K. S., 672  
 CHRISTEL, G., 676  
 CHRISTIANSEN, W. G. O., 550  
 CHUGAEV, L. (see TSCHUGAEFF)  
 CIMMINO, R., 641  
 CIVETTA, A., 631  
 CLAASZ, M., 680  
 CLARK, C. M., 576  
 CLARK, E. P., 627, 675  
 CLARK, G. W., 558  
 CLARK, H. W., 545, 640  
 CLARK, W. M., 199, 202, 216, 497, 533, 605  
 CLARKE, W. F., 626  
 CLAUDIUS, M., 539  
 CLAUSMANN, P., 599  
 CLENNELL, J. E., 630, 681  
 CLIFFORD, W. M., 563  
 CLOUD, T. C., 554  
 COFFIN, H. W., 688  
 COHEN, B., 487, 490, 491, 603  
 COHEN, I., 597  
 COHEN, J. B., 640, 651, 696  
 COHENTVAERT, D. G., 710  
 COHN, R., 615  
 COLE, W. H., 565  
 COLEMAN, 511  
 COLLARDEAU, 572  
 COLLINS, R. J., 542, 604  
 COLLINS, W. D., 279, 281, 632  
 COLLIP, J. B., 627, 675  
 COMBES, A., 245, 614  
 CONGDON, L. A., 562  
 CONELL, S. J. B., 626  
 CONNELL, W. B., 154, 156, 595  
 COOK, F. C., 589  
 COOKE, J. H., 620  
 COOPER, A. J., 551, 581, 622, 717  
 COOPER, L. H., 557  
 COOPER, S. R., 571, 584, 619  
 COQUILLAT, A., 658  
 COREY, R. B., 114, 118, 544  
 CORI, C. F., 675, 692  
 CORI, G. T., 675, 692  
 CORLEY, R. C., 592, 712  
 CORNWALL, H. B., 545  
 CORPER, H. J., 565  
 CORRADI, R., 643  
 COWLEY, R. C., 599  
 CRAWFORD, W. G., 381-3, 702  
 CREIGHTON, M., 675  
 CRIBB, C. H., 556  
 CRIBIER, J., 552  
 CRISMER, L., 334, 608  
 CRISTOL, P., 598, 668, 713  
 CROLL, H. M., 690  
 CRONER, F., 630  
 CROOKES, W., 573  
 CROUTER, C. Y., 675, 691, 692  
 CRUM, W., 628  
 CRUTZ, O., 616  
 CSONKA, F. A., 537, 566, 593, 688, 690  
 CULLEN, G. F., 51, 455, 492, 493, 511, 512, 604, 606, 607, 691  
 CULLUM, M. L., 616  
 CUNY, L., 133, 554, 663  
 CURRIE, A. N., 584  
 CURTMAN, L. J., 710  
 CYVAN, L. L., 669

## D

DAKIN, 466

- DALE, H. H., 555  
 DARIC, J., 674  
 DAUPHINEE, J. A., 606  
 DAVIES, J., 613  
 DAVIS, C. W., 577, 646  
 DAVIS, D. M., 707  
 DAVIS, G. E., 573  
 DAVISSON, B. S., 548  
 DAVY, E. W., 650  
 DAY, H. A., 610  
 DAY, H. D., 579  
 DEBIERNE, A., 248, 615  
 DÉBOURDEAUX, L., 672  
 DE CHALMOT, G., 599  
 DEERING, W. H., 545  
 DEFAYE, 565  
 DEFFINS, 586, 590  
 DEFURNEL, H., 641  
 DEHMS, F., 572, 581  
 DEHN, W. M., 84, 88, 472, 559, 567, 598,  
     600, 603  
 DELAVILLE, M., 713  
 DELORT, M., 605  
 DELPY, M., 594  
 DEMICHEL, A., 574  
 DEMOUSSY, E., 583  
 DENIGÈS, G., 320, 334, 540, 558, 570, 571,  
     583, 608, 632, 635, 644, 645, 651, 654  
 DENIS, W., 429, 437, 438, 444, 455, 502, 508,  
     523, 527, 528, 537, 539, 544, 547, 548, 555,  
     566, 585-7, 589-92, 600, 605, 621, 627,  
     659-62, 665, 667, 672, 673, 691, 704, 705,  
     707-9, 712, 716  
 DENISON, R. B., 563  
 DENNIS, L. M., 111  
 DENNSTEDT, M., 685  
 DENTLER, M. L., 587, 592  
 DESCH, C. H., 247, 615  
 DESEÜ, D. v., 619  
 DESHAYES, V., 628  
 DETONI, G. M., 567, 626  
 DEUSSEN, E., 693  
 DIBBIN, W. J., 557  
 DIECKMANN, T., 702  
 DIÉNIERT, F., 683  
 DIETERICH, K., 716  
 DIMMITT, F. W., 586, 590  
 DINGLER, 560  
 DISCHE, Z., 559  
 DITTOE, W. H., 159  
 DITTRICH, M., 168, 569, 631  
 DOAN, F. J., 621  
 DOBBIN, L., 631  
 DODDS, E. C., 597  
 DOISY, E. A., 264, 346, 457, 586, 591, 661,  
     671, 672, 684, 710  
 DOLD, H., 578  
 DOMOGALLA, B. P., 544, 596, 604  
 DONKIN, W. F., 630  
 DONNAN, F. G., 616  
 DORING, 193  
 DOSNE, P., 580  
 DOUGLAS, W. F., 608  
 DOVEY, E. R., 618  
 DOWNINWIRTH, A. L., 216  
 DOWSETT, 194  
 DOWZARD, E., 557  
 DROSE, J., 583  
 DROST, J., 624  
 DROWN, T. M., 659  
 DRUSHEL, W. A., 607  
 DUBIEF, J., 612  
 DURIN, H., 556, 626, 667  
 DUBOIS, W. L., 679  
 DUBOSCQ, J., 10, 11, 32, 38, 40, 42, 43, 112  
 DUBRUNAUT, 600  
 DUCLAUX, J., 95  
 DUFTY, L., 629  
 DUGGAN, W. F., 692  
 DUMITRESCU, 631  
 DUNLAP, 401, 412, 413  
 DUNNICLIFFE, H. B., 584  
 DUNNINGTON, F. P., 700  
 DUPRAY, M., 564  
 DUPRÉ, A., 560  
 DURIEN, E., 594  
 DUTCHER, R. A., 675, 691  
 DUYK, M., 630
- E
- EAGLES, B. A., 466, 588, 596, 601, 713  
 EASTICK, J. J., 617  
 EASTLACK, 96  
 EATON, E. P., 672  
 ECK, P. N. VAN, 166, 560, 618, 657, 671  
 EDEN, T., 605  
 EDGAR, G., 3, 97, 258, 415, 417, 459, 519,  
     587, 592, 597, 626, 685  
 EDMOND, H. D., 566  
 EFIMOV, V. V., 666  
 EGELENG, C. G., 255, 623  
 EGERER, G., 548, 661

- EGGERTZ, 10, 13, 14, 559, 560, 561, 581  
 EGGERTZ, M. W., 694  
 EGGERTZ, V., 693, 694  
 EIJKMAN, F. H., 574  
 EISENHARDT, W., 690  
 EKKERT, L., 648  
 ELION, H., 679  
 ELLMS, J. W., 158, 159, 564, 716  
 ELSDON, G. D., 547, 623, 645, 655  
 ELSTON, C. M., 697  
 ELVEHJEM, C. A., 491, 620  
 ELVOVE, E., 411, 540, 550, 653, 667  
 EMMERLING, O., 546  
 EMMET, A. D., 585, 588, 598  
 EPSTEIN, A. A., 687, 688  
 ERDMANN, C. C., 589, 707, 708  
 ERDMANN, H., 652  
 ERUST, E., 611  
 ESCHBAUM, F., 633  
 ESCHLE, O., 687  
 ESSINGER, R., 674  
 ESTES, C., 439, 440, 670, 716  
 ETS, H. N., 555  
 EVANS, B. S., 123, 125, 167, 169, 256, 551, 569, 572  
 EVANS, C. L., 555  
 EVANS, F. A., 691  
 EVERS, N., 547, 618  
 EWAN, T., 697  
 EWERS, E., 615
- F
- FABER, P., 701  
 FÁBIÁN, L., 693  
 FABINYI, R., 572, 635, 636  
 FAILYER, G. H., 359-64, 677  
 FAIRHALL, L. T., 258, 263, 298, 299, 302, 638  
 FALES, H. A., 1, 216, 346, 721  
 FALISI, J. C., 57  
 FALK, K. G., 580, 587, 591  
 FARADAY, M., 91, 98  
 FARCY, L., 642, 643  
 FARMER, C., 546, 659, 707  
 FARMER, C. J., 500, 502, 504-6, 513, 547, 659  
 FARRINGTON, E. D., 672  
 FASAL, H., 703  
 FAUCONNIER, P., 254, 624  
 FAUL, M., 636  
 FEHLING, 433, 434  
 FEIGL, F., 558
- FEIGL, J., 671  
 FIELDSBERG, E., 703  
 FELLEBERG, T. VON, 540, 542, 575, 612, 716  
 FELTY, A. R., 606  
 FERRIS, W. S., 15, 266, 269, 627  
 FEX, J., 567  
 FIEBER, R., 637  
 FILIPPI, E., 556  
 FINE, M. S., 585, 587, 589, 590, 686, 689, 708  
 FINNER, L. L., 712  
 FISCHER, 97, 99  
 FISCHER, E., 609  
 FISCHER, F., 649  
 FISHER, H. L., 585, 589  
 FISKE, C. H., 348, 544, 547, 549, 605, 659, 660, 672, 675, 707  
 FLEURY, P., 256, 419-21, 580, 599, 624, 691  
 FLORENTIN, D., 672  
 FOERSTER, F., 677  
 FOIX, A., 602  
 FOLIN, O., 34, 59, 60, 161, 162, 349, 429, 437, 438, 445-8, 451, 452, 454, 455, 457, 462, 463, 466, 467, 469-71, 481, 482, 500, 502, 504-6, 508, 510, 511, 513-15, 518-25, 527, 528, 537, 539, 544-9, 555, 584-92, 595, 621, 659-62, 665, 667, 689, 690, 693, 703, 705-7, 709, 711, 712, 716  
 FOLKARD, C. W., 681  
 FOLTZ, A., 216  
 FORAILONG, R., 686  
 FORD, F., 548, 661  
 FORESTIER, H., 629, 632  
 FORSCHBACH, 506, 686  
 FOSTER, G. L., 690  
 FOSTER, M. D., 279, 281, 632  
 FOSTER, M. L., 611, 683  
 FOSTER, N. B., 585, 589  
 FOULGER, J. H., 366, 368, 369, 683  
 FOWLER, 309  
 FOWWEATHER, F. S., 620  
 FOX, C. J. J., 331  
 FOX, F., 639  
 FOX, F. W., 567  
 FOXWELL, G. E., 307, 548  
 FRABOT, C., 704  
 FRANCIS, C. K., 154, 156, 595  
 FRANÇOIS, M., 540  
 FRANKE, E., 462, 522, 712  
 FRANKEL, E. M., 677

FRÄNKEL, S., 538, 703  
 FRANKFORTER, G. B., 326-8, 575, 665  
 FRANKLAND, E., 545  
 FREAK, G. A., 125, 550  
 FRED, E. B., 544, 596, 604  
 FREDERICK, R. C., 549, 647  
 FREED, M., 710  
 FRESH, G., 679  
 FRERICH, G., 306, 636, 642  
 FRESSENIUS, W., 574, 679, 681  
 FREUND, H., 579  
 FREYER, F., 679  
 FRIBOURG, C., 701  
 FRIEBEL, G., 659  
 FRIEDEL, R. K., 562  
 FRIEDEN, A., 92, 96  
 FRIEDENTHAL, H., 617  
 FRIEDHEIM, C., 697  
 FRIEDLÄNDER, 110, 115  
 FRIEDRICH, O. VON, 548, 636, 661  
 FRIEND, H., 539, 714  
 FROST, E. C., 555  
 FROWEIN, B., 539  
 FUEAT, E., 575  
 FULLER, C. H. F., 584  
 FUNCK, A. D., 635  
 FUNK, A., 547, 565, 575, 600, 609, 610, 617,  
     621, 623, 645, 655, 687, 709  
 FUNK, C., 703  
 FURLONG, J. R., 595  
 FURMAN, N. H., 216  
 FÜRTH, O. VON, 563, 703, 706

## G

GAD-ANDERSON, K. L., 707  
 GAEBLER, O. H., 588, 593, 692  
 GALANOS, S., 592  
 GALBRAITH, 561  
 GALBRAITH, W., 560  
 GALLAGHER, C. D., 689  
 GALLINKAMP, 574  
 GAMBLE, J. L., 567, 587, 592  
 GANGLER, F. A., 671  
 GANS, 513  
 GARDNER, J. A., 567  
 GARN, W. VON, 365, 681  
 GARRATT, F., 166, 568  
 GARREAU, Y., 690  
 GASCARD, 635  
 GAUSS, H., 636  
 GAUTIER, A., 386, 599, 701

GAYON, U., 540  
 GEELMUYDEN, H. C., 546, 641, 653  
 GEORGES, L., 635  
 GERET, L., 591  
 GERHARD, F., 615  
 GERICKE, W. F., 315, 646  
 GERRANS, H., 135, 556  
 GERSDORFF, C. E. F., 596, 703, 704, 706  
 GETTLER, A. O., 586, 590, 591, 660, 710  
 GIANNETTI, G., 573  
 GIBBS, H. D., 680  
 GIBSON, R. B., 670  
 GIES, W. J., 677  
 GIETELING, H., 673  
 GILBERT, M., 663, 692  
 GILL, A. H., 641, 651  
 GIMMINGHAM, C. T., 647  
 GIOLITTI, F., 697  
 GITTLEMAN, I., 675  
 GIVENS, M. H., 585, 590, 709, 710  
 GLADSTONE, J. H., 615, 699  
 GLASSMANN, B., 693  
 GLASTONE, S., 698  
 GODDARD, J., 555  
 GOECKEL, H. J., 538, 592  
 GOETZ, 628  
 GOIFFON, R., 668, 685  
 GOLDSBROUGH, H. A., 125, 550  
 GOLDSCHIEDER, I., 621  
 GOLDSCHMIDT, S., 587, 592  
 GOLUB, W., 663  
 GORTNER, R. A., 427, 428, 631, 705  
 GÖTHE, F., 618  
 GOTTFRIED, A., 631  
 GRABE, 659  
 GRAHAM, T., 97  
 GRAHAM, V. A., 596, 690, 704, 706  
 GRAM, H. C., 606, 607  
 GRANDVAL, A., 639  
 GRANT, N. S., 355, 676  
 GRANT, W. G., 697  
 GREATHOUSE, L. H., 273-8, 632  
 GREAVES, J. E., 642, 643, 645  
 GREEF, 153  
 GREENE, C. H., 587, 662, 708  
 GREENE, C. W., 587, 661  
 GREENWALD, I., 457, 585, 587, 589, 591,  
     593, 660, 661, 667, 671, 674, 675, 692, 713  
 GREGG, R., 621  
 GRÉGOIRE, A., 143, 270, 343, 558, 627, 672  
 GREGORY, A. W., 394, 396, 616, 714

GRIESS, P., 310, 649, 650, 664  
 GRIGAUT, A., 565, 626, 661, 711  
 GRIGGI, G., 615  
 GRIMALDI, C., 676  
 GRINDLEY, H. S., 584, 585, 588, 598, 659  
 GROBERT, J. DE, 679  
 GROBERT, S. DE, 679  
 GROSS, A., 680  
 GROSS, J., 457, 593, 674, 675, 692, 713  
 GRÜNHUT, L., 645, 679, 681  
 GRÜTZNER, P. VON, 575, 666  
 GUERBET, 617  
 GUEVIN, F., 661  
 GUILLAUMIN, C. O., 712  
 GUION, C. M., 709  
 GULICK, A., 547, 660  
 GUNNING, 500  
 GÜNSBERG, R., 573  
 GÜNTHER, T., 575  
 GUY, R. A., 690  
 GYÖRGY, P., 674

## H

HAAN, J. DE, 577  
 HAAS, F., 631  
 HAAS, G., 706  
 HAASE, L. W., 649  
 HACKL, O., 569, 571, 638, 699  
 HADEN, R. L., 445, 593, 662, 663, 691, 692, 712  
 HADFIELD, 561  
 HAESSLER, F. H., 558, 671  
 HAGER, H., 564, 639, 650  
 HAHN, F. V. VON, 685, 691  
 HAHN, Y. D., 712  
 HALDANE, 488  
 HALDANE, J., 603  
 HALE, C. F., 559  
 HALE, W., 600  
 HALL, C. C., 557  
 HALL, F. P., 216, 632  
 HALL, R. D., 383, 701  
 HALL, W. T., 548, 551, 657, 698, 702  
 HALPHEN, G., 135, 556  
 HALSKE, 572  
 HALVERSON, 457  
 HAMILTON, B., 607, 675  
 HAMMETT, F. S., 264, 266, 555, 586, 587, 590, 592, 627, 673, 710  
 HAMMETT, L. P., 109, 113, 114, 118, 543, 660

HAND, P. G. T., 666  
 HANKE, M. T., 551, 604, 605, 610, 705, 706  
 HANTZSCH, 88  
 HANTZSCH, A., 247, 615  
 HANZLIK, P. J., 542, 604, 680  
 HARCOURT, A. G. V., 253, 623  
 HARDEN, A., 637  
 HARDER, O. E., 438, 664, 716  
 HARDING, V. J., 544, 588, 592, 593, 610, 660, 662, 708  
 HARDY, C. R., 583, 618  
 HARDY, H., 253, 582, 615, 622, 633, 700  
 HARDY, H. J., 695  
 HARMAN, K. E., 55, 57, 61, 450, 500, 559  
 HARNED, B. K., 663, 692  
 HARPER, H. J., 647  
 HARPUDER, K., 712  
 HARRISON, A. P., 320, 647, 685  
 HARROW, G., 640  
 HARRY, F. T., 679  
 HART, E. B., 491, 620, 673, 674  
 HARTING, P., 613  
 HARTMAN, F. A., 472, 559, 600  
 HARTRIDGE, H., 577  
 HARTWIG, L., 631  
 HARVEY, A., 618  
 HARVEY, S., 433, 545, 573, 622, 679  
 HASELHORTH, G., 553  
 HASKINS, H. D., 603, 706  
 HASTINGS, A. B., 493, 606, 607, 673, 674  
 HATFIELD, W. D., 121, 122, 543  
 HATLEHOL, R., 689  
 HAUN, F., 647  
 HAUSER, S. J., 158, 159, 564  
 HAWK, P. B., 348, 353, 451, 457, 478, 480, 511, 545, 546, 550, 552, 567, 588, 593, 601, 627, 659, 663, 668, 675, 678, 693, 706, 708, 713  
 HAWKINS, J. A., 492, 606  
 HAWLEY, H., 556  
 HAYASHI, T., 690  
 HAZEN, A., 318, 545, 579, 640  
 HEATH, F. H., 610  
 HEATH, G. L., 176, 177, 582  
 HEATH, R. F., 583  
 HEBEBRAND, A., 556  
 HEBERT, A., 623  
 HECKSCHER, H., 599  
 HEHNER, O., 10, 14, 15, 573, 585, 588  
 HEIDENHAIN, H., 423, 426, 664  
 HEIDUSCHKA, A., 636

- HEIKE, W., 714  
 HEIM, F., 623, 654  
 HEINE, 581  
 HEINRICHS, H., 619  
 HEINTZ, W., 680  
 HEINZELMANN, A., 282, 633  
 HEINZELMANN, G., 679  
 HELLER, 600  
 HELLER, H., 664  
 HELLIGE, F., 575  
 HELLMAN, C. G., 562  
 HELMAN, F. D., 675  
 HELMER, O. M., 594  
 HEMINGWAY, A. J., 624  
 HEMPEL, H., 542  
 HENDRIX, B. M., 600, 607  
 HENES, E., JR., 565  
 HENNINGSEN, C., 668  
 HERAPATH, T. J., 572, 613  
 HERAPATH, W., 613  
 HERBERT, A., 654  
 HERRERA, C. R., 670  
 HERTER, C. A., 611, 683  
 HERTING, O., 606  
 HERTRICH, M., 619  
 HERTZFELD, E., 596, 626  
 HESLINGA, J., 632  
 HESS, A. F., 675  
 HEWITT, J. T., 617, 690  
 HEWITT, T. E., 344, 345, 669  
 HEYDE, H. C. VAN DER, 549, 661, 708  
 HIBBARD, P. L., 578, 648  
 HIGGINS, H. L., 562  
 HILL, C. A., 555  
 HILL, L. A., 357, 358, 677  
 HILL, W. L., 83, 92, 109, 544  
 HILLEBRAND, W. F., 168, 568, 700, 702  
 HILLER, A., 555, 688  
 HILTNER, R. S., 439, 570  
 HIMWICH, H. E., 691  
 HINDS, J. I. D., 558, 616, 693  
 HINEGARDNER, 519  
 HINES, H., 586  
 HINES, H. M., 586  
 HINRICHS, C. G., 642  
 HINSDALE, S. J., 635, 698  
 HINTZ, E., 561  
 HIPPLE, F., 663, 708  
 HIRSCH, E. F., 607  
 HISEY, A., 97, 307  
 HUTCHCOCK, E. H., 709  
 HOBART, F. B., 571  
 HOBSON, S., 555  
 HOFFMAN, W. S., 427, 428, 693  
 HOGG, T. W., 561, 568  
 HOLLAND, P., 630, 649  
 HOLLINGS, P., 641, 653  
 HOLLY, O. M., 567, 673  
 HOLM, G. E., 705  
 HOLT, L. E., JR., 675  
 HOLVEN, 217  
 HOLWERDA, B. J., 607  
 HOMER, A., 710  
 HOMFRAY, I., 323, 324, 327, 665  
 HOOKER, S. C., 640  
 HOPKINS, E. G., 555, 559  
 HOPKINS, E. S., 620  
 HOPPE-SEYLER, 628  
 HOPPE-SEYLER, F., 603  
 HORIUCHI, Y., 556  
 HORN, D. W., 83, 88, 568, 682  
 HORN, M., 658  
 HORNE, W. D., 578  
 HOKST, F. W., 609  
 HORVÁTH, BÉLA VON, 631  
 HÖSSLY, E. H., 576  
 HÖST, H. F., 689, 710  
 HOSTETTER, J. C., 618  
 HOUTTON, 572  
 HOWE, P. E., 546, 706  
 HOWLAND, J., 558, 627, 628, 671  
 HRYNTSCHAK, T., 563  
 HUBBARD, R. S., 537, 567, 597, 662, 690, 712  
 HUBBARD, W. S., 716  
 HUBERT, 581  
 HUGHES, E. B., 584  
 HULTON, F., 600, 660  
 HULTON-FRANKEL, 57  
 HUME, H. V., 691  
 HUNDESHAGEN, F., 346  
 HUNT, A. E., 629  
 HUNTER, A., 585-7, 590, 591, 598, 606, 703, 706, 709, 710  
 HUNTER, G., 466, 563, 596, 601, 610, 713  
 HURLEY, W. B., 10, 21, 22, 110, 403, 575  
 HUSSEY, R. E., 450, 502  
 HÜTTIG, G. F., 700  
 HÜTTNER, C., 179, 180, 239, 304, 571, 583, 618, 637  
 HUYSE, A. C., 609  
 HYVERT, G., 574



## I

- ILOSVAJ DE NAGY ILOSVA, L., 406, 538, 545,  
650, 651  
INCE, W. H., 679  
INGVALDSEN, T., 590  
IRISH, O. J., 454, 675  
ISAACS, M. L., 161, 162, 333, 366, 564, 609,  
683  
ISAACSON, V. I., 600  
ISHAM, R. M., 701  
IVANOV, V. N., 257, 624, 678  
IVES, F. E., 574

## J

- JACKSON, D. D., 314, 684, 693, 704  
JACKSON, E., 608, 700  
JACKSON, H., JR., 538, 711, 712  
JACOBSON, C., 547  
JACQUELAIN, 581  
JAFFÉ, M., 457, 472, 518, 588  
JAHR, H. M., 548, 689  
JANDRIER, 651  
JANKE, A., 608  
JANNEK, J., 681  
JANNEY, N. W., 586  
JARDIN, F., 631  
JÄRVINEN, K. K., 543, 551, 552, 584, 625,  
638, 687, 700, 718  
JENDRASSIK, L., 675  
JERSEY, V., 662  
JOHN, H. J., 444  
JOHNS, C. O., 151, 153, 154, 595, 705  
JOHNSON 216, 472  
JOHNSON, A. E., 640, 644  
JOHNSON, C. M., 14, 149, 150  
JOHNSON, G., 539, 686  
JOHNSON, M. O., 154, 595  
JOHNSTONE, W., 639  
JOLLES, A., 669, 682  
JOLLES, A. F., 614, 616  
JONAS, L., 691  
JONES, 298, 299  
JONES, A. J., 552  
JONES, C. M., 713  
JONES, C. O., 555  
JONES, D. B., 506, 703-6  
JONES, E. G., 173, 175, 571  
JONES, E. W. T., 541  
JONES, J. H., 673, 674  
JONES, L. A., 577  
JONES, M. R., 672

- JONES, O. M., 608  
JONGBLOED, F., 711  
JORISSEN, 679  
JOSEPHSON, K., 542  
JUNG, W., 282, 633  
JÜPTNER, 695  
JÜRGENS, B. H., 651

## K

- KAHLENBERG, L., 567  
KAHN, B. S., 559  
KAHN, G., 608  
KAHN, R. L., 660  
KÄMMERER, H., 603  
KANTOR, J. L., 677  
KAPSINOW, R., 714  
KARELITZ, S., JR., 603, 691  
KARR, W. G., 454, 478, 592, 605, 662, 692,  
710  
KARSNER, H. T., 547, 659, 707  
KASANOF, 344  
KAST, L., 566  
KASTLE, J. H., 605, 653  
KEATES, 581  
KEELER, H. R., 604, 607  
KEELER, L. M., 711  
KEHOE, R. A., 258, 261-3, 626  
KEKULÉ, A., 638  
KELER, H. VON, 219, 615  
KELLEY, W. P., 645  
KEMPSTER, H. L., 598  
KENDALL, L. M., 314, 684  
KENNEDY, R. P., 604  
KENNICOTT, 10, 21, 110, 574  
KENT, G. H., 565  
KENT-JONES, D. W., 217  
KERN, 560  
KERSTING, R., 638  
KILLIAN, J. A., 578  
KING, J. F., 573  
KING, J. H., 605  
KING, W. J., 285-7, 291, 292, 578, 634  
KINGSBURY, F. B., 710  
KINNICUTT, L. P., 158  
KIPP, H. A., 567  
KISLAKOWSKY, 614  
KJELDAHL, 500  
KLECKI, V. v., 714  
KLEINER, I. S., 577, 600, 603, 689, 690, 708  
KLEINMANN, H., 11, 45, 47, 48, 51, 112, 579,  
634, 663, 671, 715

- KLEMPERER, R. L. VON, 575  
 KLETT, R. E., 29, 51, 577  
 KLOSTERMANN, M., 626  
 KLUT, H., 617, 630  
 KNAPP, A. W., 541, 580, 623  
 KNIEDER, 570  
 KNIGHT, G. W., 678  
 KNIGHTS, J. W., 639, 668  
 KNORR, L., 598  
 KNOWLES, H. B., 114, 117, 543  
 KNUDSON, A., 566, 567, 672  
 KOBER, P. A., 11, 28, 29, 32, 70, 75, 77, 78, 103, 577  
 KOCH, F. C., 447, 450, 502, 506, 508, 549, 636, 662  
 KODAMA, S., 539  
 KOEHLER, A. E., 555, 712  
 KOENIG, P., 165, 568  
 KOENIGSBERGER, J., 575, 576  
 KOESSLER, K. K., 551, 604, 610, 705, 706  
 KOHOUT, J. F., 562  
 KOLLIS, A. C., 681  
 KOLTHOFF, I. M., 216, 334, 335, 548, 595, 606, 646-8, 656-8, 717  
 KÖNIG, F., 546  
 KÖNIG, F. J., 615, 652  
 KONINCK, L. L. DE, 546, 633  
 KOPP, E., 316, 638, 649  
 KOPPESCHAAR, W. F., 611  
 KOPPMAYER, M., 628  
 KOSTJAMIN, N. N., 641  
 KRAMER, B., 264, 265, 558, 627, 628, 673, 688  
 KRAMER, J. G., 586, 591, 661  
 KRASILEWSKY, S., 567  
 KRAUS, I., 703  
 KRAUSS, R. B., 612  
 KREGTEN, Y. R. A., VON, 664  
 KRISTELLER, L., 707  
 KROPACSV, S., 608  
 KROPP, A., 715  
 KRÜSS, G., 614  
 KRÜSS, H., 574  
 KUGELMASS, I. N., 673  
 KÜHN, 623  
 KUNDRÁT, F., 714  
 KURBATOV, 87  
 KURIYAMA, S., 539, 542, 688  
 KÜRTHY, L., 554, 555  
 KÜSPERT, F., 406, 538  
 KUTTNER, T., 576, 591
- L
- LABILLARDIÈRE, 572  
 LACHS, H., 617  
 LAFORE, J., 643  
 LAIDLAW, P. P., 139, 142, 558  
 LAJOUX, H., 639  
 LAMB, A. B., 30  
 LAMPADIUS, W. A., 570  
 LAMPE, B., 647  
 LAMPITT, L. H., 584  
 LANDER, G. D., 594  
 LANGE, C., 539  
 LANGE, N. A., 612  
 LANGSTROTH, L., 661  
 LAPICQUE, L., 614  
 LAPORT, X., 676  
 LAPORTE, C. E., 133, 554  
 LAROSE, E., 143, 558  
 LAUTERBACH, F., 552  
 LAVIALLE, P., 595  
 LAWTON, V. L., 57  
 LEATHER, J. W., 616  
 LEBERMAN, F., 578, 604  
 LE CHATELIER, H., 562  
 LEDEBUR, A., 628  
 LEDEBUR, H., 561  
 LEE, F. A., 610  
 LEEDS, A. R., 560, 573, 650  
 LEFFMANN, H., 659  
 LEHMAN, E. P., 565  
 LEHMANN, K. B., 637  
 LEIBOFF, S. L., 567  
 LEITER, L., 610  
 LELEY, L., 285  
 LEMAIRE, M., 629  
 LENHER, V., 381, 382, 383, 702  
 LENK, E., 537  
 LENNOX, W. G., 607, 663, 713  
 LENORMAND, C., 665  
 LEO, K., 676  
 LEPIERRE, C., 669  
 LEROY, G., 682  
 LEROY, G. A., 564  
 LETTS, E. A., 645, 656  
 LEUBA, J., 83  
 LEVENE, P. A., 703  
 LEVERKUS, 638  
 LEVINE, V. E., 636  
 LEVY, 148  
 LEVY, L., 551, 553, 580, 699, 700, 714  
 LEWIS, D. S., 689

- LEWIS, H. B., 555, 591-3, 596, 600, 660, 662, 663, 667, 710, 712, 713  
 LEWIS, H. F., 550  
 LEWIS, J. H., 539  
 LEWIS, P. A., 612  
 LEWIS, R. C., 472, 576, 687  
 LEY, H., 615  
 L'HÔTE, L., 581  
 LICHTIN, A., 619  
 LIEBEN, F., 703  
 LIEBERMANN, L., 253  
 LIEBRICH, A., 254, 622  
 LIFSCHÜTZ, I., 553, 565  
 LIMPRICHT, R., 680  
 LINCOLN, A. T., 369-71, 669  
 LINDER, G. C., 555  
 LINDFIELD, J. H., 617  
 LINDO, D., 563, 630, 650  
 LINDSAY, W. G., 373, 696  
 LINDT, V., 296, 297, 637  
 LINTNER, C. J., 677  
 LIPMAN, C. B., 645  
 LISCI, F., 617  
 LITTLE, L. D., 570  
 LIVERSEEGE, J. F., 541  
 LIZINS, J. L., 672  
 LOCKE, A. P., 663  
 LOCKEMANN, G., 594  
 LOEB, J., 92  
 LOEBEL, R. O., 691  
 LOHMANN, K., 675  
 LOMBARD, M., 643, 655  
 LONG, J. H., 598  
 LONG, M. L., 592  
 LONG, W. L., 714  
 LONSTEIN, I., 675  
 LOONEY, J. M., 595, 596, 703, 704, 706  
 LORBER, L., 620  
 LOSANA, L., 342, 672  
 LOVIBOND, J. W., 2, 10, 11, 573-5, 579, 580, 684  
 LOVIBOND, T. W., 573  
 LÖWE, F., 575  
 LUCAS, M., 253, 254, 373, 582, 622, 637, 696  
 LUDEK, G., 566  
 LÜHPIG, H., 630, 631  
 LUNDELL, G. E. F., 114, 117, 543  
 LUNGE, G., 219, 228, 234, 615, 641, 650, 651, 653  
 LÜSCHER, E., 703  
 LUTZ, R. E., 718  
 LWOFF, A., 641, 651  
 LYFORD, E. F., 401, 412, 413, 541  
 LYMAN, H., 143, 145, 146, 558  
 LYMAN, J. F., 586, 591  
 LYONS, A. B., 540, 600  
 LYONS, E., 620  
 M  
 MAAG, O. L., 292, 294, 634  
 MACALLUM, A. B., 546, 547, 659, 707, 709  
 MCAMIS, A. J., 607  
 MCCABE, C. R., 377-9, 388-91, 393, 702, 715  
 MCCANCE, R. A., 693  
 MCCLEAN, A. P. D., 563  
 MCCLENDON, J. F., 493, 494, 497, 498, 605, 606, 608, 612  
 MCCLURE, C. W., 567  
 MCCLUSKEY, K. L., 674  
 MCCOLLAM, C. H., 292, 294, 634  
 MCCrackan, R. F., 55, 57, 58, 61, 450, 500, 531, 532, 555, 559, 632  
 MCCRUDDEN, F. H., 143, 145, 146, 566, 586, 590, 600  
 MCDANELT, L., 687, 688  
 MCELLROY, W. S., 603, 634, 661, 708  
 MCGUIGAN, H., 597, 688, 689  
 MCGUIRE, G., 587, 591  
 MCILVAINE, T. C., 557  
 MCINERNEY, T. J., 608  
 MCINTOSH, J. F., 606, 607, 673, 674  
 McLAUGHLIN, L., 593  
 MACLEAN, R. M., 544, 610, 660  
 MACLEOD, A. G., 711  
 MACLEOD, G., 593  
 MACLEOD, J. J. R., 686, 687  
 McMEekin, T. L., 447, 450, 502, 506, 508, 549, 636, 662  
 McQUARRIE, I., 607  
 McRAE, H. C., 642  
 MAGNANINI, G., 614  
 MAI, C., 635  
 MAI, J., 551  
 MAILLARD, L., 714  
 MAIN, E. R., 663  
 MAIWEG, H., 539  
 MALETTE, J., 630  
 MALOWAN, S. L., 634  
 MANDELIN, K. F., 542  
 MANN, G. R., 617, 699  
 MANNEBACH, O., 577

- MANNHEIM, E., 306, 636  
 MAQUENNE, L., 583, 619  
 MARCANTONIO, M., 697  
 MARCILLE, R., 641, 642  
 MARKER, J., 586  
 MARQUEVROL, 656  
 MARRIOTT, W. MCK., 234, 558, 562, 616, 627, 671  
 MARSHALL, E. K., JR., 455, 707  
 MARSHALL, H., 271, 272, 630  
 MARSHALL, J. T. W., 528  
 MARSHALL, W. E., 611  
 MARTIN, H., 659  
 MARTLAND, M., 674  
 MARTZ, 216  
 MARTZ, F., 537  
 MARUOKA, K., 707  
 MASON, E. H., 600, 689  
 MASON, W. P., 545, 641  
 MATEJKA, J., 619  
 MATHEWS, A. P., 57  
 MATHIEU, L., 618, 619  
 MATTHEWS, S. A., 547  
 MAURER, E., 562  
 MAURIAC, P., 565  
 MAVER, M. E., 621  
 MAXSON, R. N., 601  
 MAY, C. E., 703  
 MAYER, O., 617, 618  
 MEADE, G. P., 559  
 MEARS, B., 450, 502  
 MEAURIO, V. L., 715  
 MECKLENBURG, A. W., 576  
 MECKLENBURG, W., 376, 610, 704  
 MEDALIA, L. S., 606  
 MEDINGER, P., 670  
 MEEK, W., 674  
 MEERBURG, P. A., 255, 624, 658  
 MEIMBERG, E., 580, 581  
 MEISLING, A. A., 574  
 MELDRUM, R., 397, 399, 624, 718  
 MELDRUM, W. B., 30  
 MELLANBY, E., 588  
 MELLON, M. G., 685  
 MELLOR, J. W., 172, 237, 570, 616, 702, 715  
 MENAUL, P., 698  
 MENDEL, B., 621  
 MENDEL, L. B., 589  
 MENNICKE, H., 652  
 MERRILL, P. W., 719  
 MERWIN, H. E., 186, 599, 701  
 MEULEN, H. TER, 634  
 MEULENGRACHT, 480, 552  
 MEULENGRACHT, E., 553, 577  
 MEYER, 490  
 MEYER, A., 554  
 MEYER, G. M., 707  
 MEYER, J., 681  
 MEYERFELD, J., 568, 617, 654  
 MEYERS, 589  
 MEYSENBURG, L. VON, 672  
 MICHAELIS, 161  
 MIDDLETON, A. R., 113, 118, 544  
 MIGNOT, A., 629  
 MILBAUER, J., 582, 637  
 MILDNER, H., 631  
 MILLAR, C. E., 671  
 MILLER, C. W., 611, 667, 670  
 MILLER, E. H., 311, 655  
 MILLER, E. M., 547  
 MILLER, F. B., 572  
 MILLER, J., 254, 625  
 MILLER, R., 604  
 MILLER, W. A., 545, 622  
 MILLIGAN, L. H., 658  
 MILLON, 431, 432  
 MILLS, E. J., 573  
 MILNE, J. M., 545  
 MILROY, J. A., 601  
 MINGAYE, J. C. II., 676  
 MINK, F., 677  
 MINOT, A. S., 587, 591, 605, 661  
 MISSON, G., 342, 375, 669, 673, 697, 698, 716  
 MITCHELL, C. A., 435, 698  
 MÜLLER, G., 694  
 MÜLLER, H. J., 580  
 MOELIER, O., 596, 703, 704, 706  
 MOERK, F., 716  
 MOERK, F. X., 641  
 MOFFATT, M. R., 257, 623  
 MOIR, 481, 601  
 MOLSTAD, R., 702  
 MONNIER, A., 163, 666  
 MONTANARI, C., 641, 679  
 MONTIGNY, W., 633, 687  
 MOOR, C. G., 582  
 MORAHT, H., 614  
 MOREAU, E., 576, 577  
 MOREAU, L., 128, 551  
 MORGAN, A. F., 542, 588, 593, 708, 713  
 MORGAN, J. J., 629, 695

MORGAN, W. V., 257, 624  
 MORGENSTERN, V., 608  
 MORGULIS, S., 548, 600, 621, 636, 675, 689  
 MORRELL, T. T., 359, 613, 628, 677  
 MORRIS, J. L., 585, 586, 590, 662, 710, 711  
 MORTIMER, E., 567  
 MORTON, 572  
 MOSENTHAL, H. O., 688  
 MOTHERWELL, H. A. B., 133, 554  
 MOUD, R., 712  
 MOULIN, A., 256, 568  
 MOUNEYRAT, A., 616  
 MOUREU, C., 407, 538  
 MOYER, J., 577  
 MUELLER, J. H., 566  
 MUIR, M. M. P., 581, 622  
 MÜLLER, 706  
 MÜLLER, A., 572, 581, 613  
 MÜLLER, G., 585, 589, 686  
 MÜLLER, H., 554, 555  
 MÜLLER, J. A., 640  
 MÜLLER, M., 545  
 MÜLLER, W., 593  
 MÜLLER-HÖSSLY, E., 576  
 MULLER, 604  
 MULLER, J. A., 602  
 MULLER, J. H., 702  
 MUMFORD, E. M., 667  
 MUMMERY, W. R., 620, 679  
 MURAOUR, H., 656  
 MURLIN, J. R., 688, 692  
 MURRAY, H. A., JR., 606  
 MURRAY, M. M., 708  
 MUSSEHL, F. E., 674  
 MUTER, 678  
 MUTHMAN, 334  
 MYER, J., 365  
 MYERS, B. A., 672  
 MYERS, R. G., 555  
 MYERS, V. C., 11, 455, 456, 472, 476, 477, 493, 533, 555, 566, 576, 577, 584, 585, 587-90, 606, 607, 661, 688-90, 708  
 MYLIUS, F., 677

## N

NAGEL, 334  
 NAKASHIMA, Y., 707  
 NASH, T. P., JR., 549, 663, 692  
 NASKE, T., 606  
 NATANSON, 218  
 NEAU, A., 600

NECKE, A., 626  
 NEITZEL, E., 559, 600  
 NELLANS, C. T., 690  
 NELSON, E. E., 587, 661  
 NELSON, V. E., 611, 684  
 NEPVEUX, F., 668  
 NESSLER, J., 10, 12, 24, 96, 306, 447, 500, 545, 581, 638  
 NEUHAUSEN, B. S., 673  
 NEURATH, F., 669, 682  
 NEUWIRTH, I., 591, 710  
 NEWCOMB, C., 593  
 NEWCOMER, H. S., 51, 482, 483, 487, 490, 577, 603  
 NEWTON, 466  
 NICHOLLS, J. R., 636  
 NICHOLS, M. L., 571, 584, 619  
 NICHOLS, M. S., 316, 647  
 NICHOLSON, E., 638, 649  
 NICHOLSON, S. T., JR., 690  
 NICOLAU, 631  
 NICOLET, B. H., 667  
 NIECE, F. E., 576  
 NIXON, I. G., 617, 658  
 NOBACK, C. V., 692  
 NOBEL, E., 703  
 NOLL, H., 641  
 NOTTBOHN, F. E., 617  
 NOVELLI, A., 658  
 NOWAK, C. A., 576  
 NOYES, H. A., 647  
 NOYES, H. M., 580  
 NOYES, W. A., 700  
 NUTTING, 2, 11  
 NYE, L. L., 672  
 NYMAN, E., 618

## O

O'CONNOR, M., 607, 663, 713  
 O'CONNOR, M. F., 713  
 ODINOT, L., 571  
 ODLING, W., 573  
 OELSNER, A., 647, 656  
 OERUM, H. P. T., 616  
 OGILVIE, J. P., 617  
 OKADA, S., 690  
 OKEY, R., 603, 611, 626, 692  
 OLSZEWSKI, W., 578, 704  
 OPPENHEIMER, E., 557  
 OPPENHEIMER, R., 591  
 ORELKIN, B., 249, 618

ORLOW, N. A., 677  
 ORMANDY, R., 640, 651  
 ORR, A. P., 549  
 ORR, J. B., 590  
 ORR, T. G., 593, 663, 692  
 OSBURN, D. F., 542, 588, 593, 708, 713  
 OSER, B. L., 478, 692  
 OSGOOD, E. E., 603  
 OSMOND, F., 668  
 OSMOND, M., 628  
 OSSIAN, H., 613  
 OSTERBERG, E., 525, 526, 588, 593, 689, 690  
 OSTWALD, W., 97-9  
 OWEN, R. G., 621

## P

PACINI, A. J. P., 621  
 PAEPE, D. DE, 652  
 PAGLIANI, S., 613  
 PAGNOUL, A., 642  
 PALLADIN, A., 703  
 PALMER, J. D., 635  
 PALMER, L. S., 598, 717  
 PALMER, R. M., 126, 551  
 PALMER, W. W., 603, 688, 711, 712  
 PARAVICINI, 562, 575  
 PARKER, C. E., 570  
 PARKER, J. S., 560  
 PARKS, 575  
 PARRY, J., 629  
 PARSONS, L. B., 608  
 PARSONS, L. W., 664  
 PASSAMANECK, E., 55, 57, 61, 450, 500, 531, 532, 559, 632  
 PASSERINI, N., 670  
 PATON, F. J., 691  
 PAUL, J., 540  
 PAVELKA, F., 558  
 PAYEN, 686  
 PAYNE, G. A., 679  
 PAYNE, W. W., 139, 142, 558  
 PEARCE, R. G., 472, 660  
 PECK, 551  
 PEEBLES, A. R., 576  
 PELKAN, K. F., 474, 567, 667, 714  
 PELLET, H., 574, 679, 701  
 PELLINI, G., 682  
 PEMBERTON, R., 675, 692  
 PEPPER, O. H. P., 660  
 PERLZWEIG, W. A., 552  
 PERRIER, G., 642

PERVIER, N. C., 427, 428  
 PETERS, A. W., 661  
 PETERS, J. T., 693  
 PETERS, S., 628  
 PETERSEN, T., 599  
 PETERSON, W. H., 544, 596, 604  
 PETRÉN, 659  
 PETRÉN, J., 607  
 PETTIBONE, C. V. J., 546, 659, 707  
 PEIZENMAIER, K., 592  
 PHELPS, E. B., 564, 582, 646  
 PHILIP, J. C., 618, 699  
 PHILLIPS, H. J., 561  
 PHILLIPS, W. T., 133, 554  
 PICARD, 88  
 PICHARD, P., 628, 629, 641, 651  
 PICKHARDT, E. G., 684  
 PILHASBY, B. M., 540  
 PINCKNEY, R. M., 605  
 PINCUS, J. B., 673  
 PINCUSOHN, L., 548  
 PLANÈS, P., 132, 553, 608  
 PLASS, E. D., 586, 591, 627  
 PLESCH, J., 574  
 POHLAND, E., 633  
 POIROT, G., 133, 419-21, 554, 590  
 POLLACCI, E., 699  
 POLLARD, W. B., 602  
 POLLOCK, H. O., 661, 708  
 POLONOWSKI, 621  
 POMARET, M., 677  
 POPOFF, S., 216  
 POPPER, H., 559  
 PORTER, L. E., 565  
 POTTER, R. S., 646  
 POUGET, I., 546, 643, 654, 670  
 POVELL, A. R., 704  
 POWELL, A. D., 172, 571  
 POZZI, W., 624  
 POZZI-ESCOT, E., 602  
 PRANDL, O., 631  
 PRATT, D. S., 313, 643, 644  
 PRESCHER, J., 657  
 PRESTON, 480, 552  
 PREUSSE, C., 650  
 PRICE, P. H., 698  
 PRIMOT, 655  
 PRISTER, A., 195, 601  
 PROCTER, H. R., 574, 633  
 PROSCHOWSKY, P., 710  
 PUCHER, G. W., 427, 599, 610, 674, 699, 711

PUENTE, J. DE LA, 396, 717  
 PULFRICH, C., 574  
 PULSIFER, H. B., 248, 616, 697, 698  
 PUSCH, 573  
 PYRIKI, C., 584, 625, 691

## Q

QUARTAROLI, A., 609, 656

## R

RABEN, E., 346  
 RACZKOWSKI, S. DE, 601  
 RADULESCU, D., 635  
 RAIKOW, P., 642, 653  
 RAIKOW, P. N., 664  
 RAIZISS, G. W., 712  
 RAKESTRAW, N. W., 481, 482, 661, 668, 691,  
 712  
 RAM, K., 584  
 RAMAGE, H., 629  
 RAMSAY, W., 323, 324, 327, 665  
 RANGLES, F. S., 672  
 RANDOLPH, D. W., 216  
 RAQUET, D., 643  
 RATH, C., 635  
 RATHERY, 586, 590  
 RAVICH, M., 620  
 RAY, B. J., 644, 654  
 RAYLEIGH, 75  
 REA, F. W., 645, 656  
 READ, A. A., 562  
 READ, B. E., 592, 714  
 READ, T. A., 624  
 REDDROP, J., 629  
 REDFIELD, H. W., 313, 644  
 REESE, C., 583, 624  
 REICHER, K., 687  
 REIMANN, S. P., 579  
 REINOSO, E. A., 589  
 REINSCH, 123  
 REISS, J., 688  
 RÉMONT, A., 678, 679  
 REVIS, C., 679  
 RHEINECK, H., 572, 613  
 RIBAU, J., 614, 615  
 RICE, E. W., 578  
 RICH, S. W., 545  
 RICHARDS, A. N., 708  
 RICHARDS, E. H., 314, 542, 716  
 RICHARDSON, A., 608  
 RICHARDSON, F. W., 541, 641, 646, 653, 676

RICHARDSON, H. A., 651  
 RICHARDSON, H. B., 600  
 RICHMOND, H. D., 549, 636, 637, 663  
 RICHTER-QUITTNER, M., 711  
 RIDEAL, S., 323, 640, 665  
 RIDSDALE, C. H., 561, 573, 574  
 RIEGLER, E., 341, 539, 652, 670, 687, 709  
 RIEMER, A., 696  
 RIESS, G., 657  
 RIFFART, H., 601, 662, 691  
 RIGGS, L. W., 612  
 RIMINGTON, C., 674  
 RING, C. H., 3, 251, 548, 646, 656, 671, 680,  
 684, 710, 716  
 RINMAN, L., 694  
 RIVAT, G., 596  
 ROAF, H. E., 598  
 ROAKE, C. E., 564  
 ROBB, E. I., 603, 692  
 ROBERTS, N., 575  
 ROBERTSON, J. H., 97, 307  
 ROBINSON, H. W., 493, 604, 606, 607  
 ROBINSON, T. W., 561  
 ROBISON, R., 674  
 ROBSCHT, F. S., 603  
 ROBSON, W., 607  
 ROCKWOOD, R., 693  
 ROCQUES, X., 540, 550, 620  
 RODENBURG, J., 631  
 RODILLON, G., 658  
 ROE, J. H., 454, 559, 675  
 ROEKE, 605  
 ROGAI, A., 333, 609  
 ROGERS, H., 712  
 ROGERS, H. W., 114, 118, 544  
 ROGERS, J. S., 318, 643  
 ROHDE, A., 553  
 ROHLAND, P., 572  
 ROLLET, A.-P., 638  
 ROMIJN, G., 172, 309, 644, 646, 652, 654, 656  
 RONA, P., 589  
 RONNET, L., 542  
 ROOS, E., 611  
 ROSE, 511  
 ROSE, A. R., 450, 501, 663, 690  
 ROSE, E. R., 703  
 ROSE, M. S., 586, 591, 593  
 ROSE, T. K., 196, 601  
 ROSE, W. C., 583, 585, 586, 589, 590, 594,  
 709  
 ROSÉE, 608

ROSENBLOOM, J., 565  
 ROSENFELD, M., 640, 651  
 ROSENHEIM, A., 615  
 ROSENHEIM, O., 541  
 ROSENKRÄNZER, F., 376, 610  
 ROSENTHAL, F., 552  
 ROSS, E. L., 597, 688  
 ROSS, H., JR., 662  
 ROSSI, A. J., 629  
 ROST, C. O., 631  
 ROTH, C., 563, 564  
 ROTHBERG, V. E., 691  
 ROTHROCK, H. A., 675  
 ROTHWELL, C., 673  
 ROULLIER, C. A., 703  
 ROWELL, H. W., 132, 554  
 ROY, A. C., 678  
 RUINI, G., 600  
 RUNNE, H., 594  
 RUSSELL, D. W., 621  
 RUSSELL, S., 493, 494, 608  
 RUSSWURM, 641  
 RYFFEL, J. H., 621

## S

SABANEJEFF, 614  
 SABATINI, G., 676  
 SABOLEV, N., 604  
 SACHER, J. F., 631, 632, 665  
 SACHNOVSKA, A., 711  
 SACHNOVSKA, A. A., 711  
 SACKETT, G. E., 567  
 SAGAIDACHNUI, A., 620  
 SAGLIER, F., 540  
 SAHLI, H., 488, 489  
 SALLERON, 572  
 SALOMON, C., 689  
 SALVADORI, R., 682  
 SALVESEN, H. A., 563, 606, 607, 673, 674  
 SAMET, J., 674, 692, 713  
 SAMMET, C. F., 576  
 SAMNELY, F., 603  
 SANDER, F. V., 444, 712  
 SANDIFORD, K., 662  
 SANGER, C. R., 551, 635  
 SARGENT, 21, 574  
 SARGENT, C. S., 566, 586, 590, 600  
 SATO, A., 671  
 SAUERLAND, F., 680  
 SAUNDERS, L., 258, 626  
 SAYBOLT, 10

SCALES, F. M., 320, 647  
 SCANDOLA, E., 686  
 SCEZEPANIK, J., 574  
 SCHAEFFER, J. A., 617  
 SCHAEER, E., 615  
 SCHAEFFER, F., 687  
 SCHAEFFER, P. A., 589  
 SCHALL, H., 537  
 SCHAULIN, A., 603  
 SCHEFFLER, K., 552  
 SCHELLBACH, H., 631  
 SCHERINGA, K., 623  
 SCHERTZ, F. M., 563  
 SCHIDROWITZ, P., 125, 541, 550  
 SCHIFF, 407, 422  
 SCHLEGEL, J. W., 598, 608  
 SCHLESINGER, E., 575  
 SCHMIDT, E., 681  
 SCHMIDT, J. G., 540  
 SCHMIDT, M. R., 630  
 SCHMIDT, P., 626  
 SCHMITZ, H. C., 708  
 SCHMITZ, H. W., 606  
 SCHNEIDER, 594  
 SCHNEIDER, A., 546, 636  
 SCHNEIDER, L., 629  
 SCHOENMAKER, P., 543  
 SCHOETTER, W. R., 704  
 SCHOLZ, A., 575  
 SCHÖNE, E., 608  
 SCHÖNFELD, F., 721  
 SCHÖNN, 334, 377, 384, 608, 634, 700  
 SCHOTT, F., 433, 582, 680  
 SCHOWALTER, E., 631  
 SCHRAMM, E., 216  
 SCHREIBER, E., 565  
 SCHREIBER, N. E., 633  
 SCHREINER, O., 4, 7, 8, 10, 15, 18, 21, 266,  
 268, 269, 339-41, 369-71, 402, 574, 579,  
 627, 669, 682  
 SCHROEDER, H., 610  
 SCHRÖDER, R., 342, 671  
 SCHRUYVER, S. B., 700  
 SCHUBERT, H., 598  
 SCHULTZ, G., 652  
 SCHULZE, A., 406, 538  
 SCHUIZE, H., 615  
 SCHUMACHER, II, 282, 633  
 SCHUMACHER, H., 562  
 SCHUYTEN, M. C., 311, 651  
 SCHWALBE, C., 436, 699



- SCOTT, E. L., 602  
 SCOTT, R. D., 667  
 SCOTT, W. W., 99, 184, 252, 397, 543, 549, 555, 557, 562, 563, 569, 578, 584, 599, 602, 620, 625, 632, 649, 650, 702, 718  
 SCOVILLE, W. L., 409, 539, 664  
 SCRIPTURE, E. W., 216  
 SEABORNE, F. S., 355, 676  
 SEDGWICK, J. P., 710  
 SEPTON, L. B., 657  
 SEIDEL, A., 600  
 SEIDEL, W., 663  
 SEIDELL, A., 539, 612  
 SENDROY, J., JR., 493, 607  
 SERAING, G. M., 697  
 SERGER, H., 582, 680  
 SEVERIN, 596, 686  
 SEVRINGHAUS, E. L., 497, 663, 673, 708  
 SEYDA, 565  
 SEYDA, A., 615  
 SEYMOUR-JONES, R. A., 633  
 SHAFFER, P. A., 518, 586, 590, 687  
 SHARP, L. T., 645  
 SHARP, P. F., 608  
 SHARPE, W. F., 555, 559  
 SHARPLESS, F. F., 561  
 SHAXBY, J. H., 608  
 SHEAFF, H. M., 665  
 SHEARER, W. L., 216  
 SHERMAN, H. C., 680  
 SHERMAN, H. S., 680  
 SHEVKY, A. E., 597, 689  
 SHEVKY, M. C., 672  
 SHILTON, A. J., 614  
 SHMUK, A., 648  
 SHOHL, A. T., 605, 607  
 SHOUB, H. L., 646  
 SHREWSBURY, H. S., 541  
 SIEDNER, C. F., 697  
 SIEGFRIED, M., 624  
 SIEMENS, 572  
 SIEMSEN, J. A., 193, 602  
 SILBER, J., 645  
 SILVESTER, H., 645  
 SIMMONDS, C., 540  
 SIMONOT, E., 583  
 SIMPSON, G. E., 592, 667  
 SINGLETON, W., 621  
 SITSSEN, M. H. P., 538  
 SJOLLEMA, B., 588, 593, 621, 673, 703  
 SLAGLE, E. A., 216  
 SLAWIK, P., 617, 715  
 SLEMONS, J. M., 710  
 SMEATON, W. G., 21, 574  
 SMIRK, F. H., 620  
 SMITH, 363, 589  
 SMITH, A. H., 487, 490, 491, 603  
 SMITH, A. P., 639, 650  
 SMITH, E. F., 383, 613, 701  
 SMITH, G. E., 612  
 SMITH, H. L., 620  
 SMITH, L., 646  
 SMITHER, F. W., 719, 720  
 SMITT, N. K., 542  
 SMORODINTZEV, I. A., 666, 703  
 SNELL, F. D., 8, 127, 135, 178, 193, 308, 333, 373, 375, 543, 549, 552, 554, 557, 562, 564, 569, 571, 577, 580, 583, 595, 597, 599, 602, 609, 610, 619, 624, 627, 632, 637, 638, 647, 658, 664-6, 672, 677, 681, 683, 698, 702, 704, 715, 717, 718  
 SNODDY, A. O., 569  
 SNYDER, R. S., 646  
 SOKHEY, S. S., 549, 675  
 SOLA, T., 143, 270, 558, 627  
 SONDÉN, K., 685  
 SONSTADT, E., 601  
 SÖRENSEN, 199, 493  
 SOTTERY, C. T., 109, 113, 114, 118, 543  
 SOULIÉ, L. A., 538  
 SPICA, M., 679  
 SPIEGEL, I., 639  
 SPINDLER, F. G., 577  
 SPIRO, H. S., 257, 623  
 SPLITTGERBER, A., 644, 645  
 SPRENGEL, H., 638  
 SPÜLLER, J., 637  
 SPURGE, G., 133, 284, 554, 634  
 STADIE, W. C., 493, 607, 634, 676  
 STAMMER, C., 572, 573  
 STANĚK, V., 582  
 STANFORD, R. V., 547, 549, 575, 578, 598, 691  
 STANICHITCH, 631  
 STARR, H. E., 606  
 STARSKY, B., 658  
 STEAD, J. E., 560, 573  
 STEAD, M., 561  
 STEEL, M., 546  
 STEENBOCK, H., 673, 674  
 STEHLE, R. L., 606  
 STEIGER, G., 15, 186, 189, 190, 384, 575, 599

STEIN, E. H., 687  
 STEINLE, J. V., 567  
 STEPHANDIES, M., 717  
 STERN, H. T., 607  
 STEUBER, A. H., 598, 608  
 STEWART, 88, 323  
 STEWART, R., 642, 643, 645  
 STICH, 564  
 STOCK, A., 633  
 STOKES, A. W., 573  
 STOKES, H. N., 100, 101, 156, 218, 219, 222,  
 224-6, 229-33, 333, 574, 616  
 STOKES, J., JR., 608  
 STONE, F. B., 553  
 STONE, W. J., 555  
 STONER, W. H., 478, 559  
 STORF, H., 658  
 STRUVE, H., 611  
 SUBBAROW, Y., 348, 675  
 SULLIVAN, M. X., 595  
 SUMNER, J. B., 524, 547, 548, 596, 597, 660,  
 690, 692, 704, 706, 707  
 SUTCLIFFE, J. A. L., 645, 655  
 SUTTHOFF, W., 644, 654  
 SVEDBERG, A., 693  
 SVEDBERG, THE, 97, 98  
 SWANSON, W. W., 662, 678, 713  
 SWEENEY, M., 553  
 SWEENEY, O. R., 137, 557  
 SWEET, J. E., 600  
 SWOBODA, F. K., 662  
 SXCZYPINSKI, A., 693  
 SYME, W. A., 642  
 SZILÁRD, P., 480, 553

T

TAGGERT, G. C., 690  
 TAKATA, M., 685  
 TALBOT, H. P., 630  
 TAMM, A., 346, 694  
 TANKARD, A. R., 556  
 TARUGI, N., 630  
 TASHIRO, S., 553  
 TASKER, 298, 299  
 TASSILLY, E., 618  
 TATLOCK, R. R., 218, 234, 315, 614, 623,  
 642, 653  
 TATUM, A. L., 689  
 TAUB, A., 685  
 TAYLOR, A. E., 547, 589, 590, 600, 611, 660,  
 667, 670, 706, 709

TAYLOR, E. R., 560  
 TAYLOR, W. A., 198, 608  
 TEED, F. L., 354, 622  
 TERADA, Y., 674  
 TERRILL, E. H., 603  
 TESDORPF, T., 596  
 TESTONI, G., 666, 688  
 THAMANN, F., 258, 626  
 THEIS, R. C., 353, 478, 479, 481, 482, 592,  
 667, 668, 674, 711  
 THIEULIN, R., 601  
 THOBURN, T. W., 680  
 THOMAS, A. W., 92, 95, 96  
 THOMAS, P., 307, 547, 583, 668, 705, 706  
 THOMAS, W., 685, 691  
 THOMPSON, H. C., 714  
 THOMSON, A., 614  
 THOMSON, R. T., 315, 623, 642, 653  
 THORPE, T. E., 639  
 THRESH, J. C., 253, 625, 651  
 THRESH, T. C., 553  
 THRO, W. C., 578  
 THURLOW, S., 714  
 TIDY, C. M., 573  
 TIEMANN, F., 650  
 TILLMANS, J., 631, 643-6, 654  
 TISDALL, F. F., 264, 265, 558, 627, 667,  
 672  
 TISDALL, F. S., 606  
 TIZARD, H. T., 550, 610  
 TOGGENBURG, F., 575  
 TOLMAN, L. M., 402, 421, 422, 541, 599  
 TOLMAN, R. C., 578  
 TOLSTOI, E., 592, 605, 673, 691  
 TOOMBS, C., 657  
 TOUJAN, G., 538  
 TOWLES, C., 589  
 TRACY, E., 493, 494, 608  
 TRAUTMANN, W., 706  
 TRAVERS, A., 101, 387, 704  
 TREADWELL, F. P., 548, 551, 632, 657, 698,  
 702  
 TRELLES, R. A., 704  
 TRILLAT, A., 546, 716  
 TRIMBLE, H., 466, 712  
 TRIMBY, J. C., 586, 591  
 TROMMSDORFF, H., 545  
 TRÜMLER, G., 585  
 TSCHUGAEFF, L., 249, 296, 618, 637, 664  
 TUCKER, 561  
 TURCHET, 546



## SUBJECT INDEX

(*References to the Bibliography are in Italics*)

### A

- Accuracy of colorimetric methods, 7
- Acetaldehyde, determination of, 401
- Acetanilide, 537
- Acetic acid, 537
- Acetoacetic acid, 537
- Acetone, 537
- Acetylacetone method for iron, 245
- Acetylene, 538
  - determination of, 402
- Acids, determination of, boric, 133, 135
  - fatty, 474
  - nitrates in, 318
  - strength of, in aqueous solutions, 720
- Acrolein, 538
  - determination of, 407
- Adenine nucleotide, 538
- Adrenaline (Epinephrine), 538
  - determination of, 408
- Adsorption complex, 95
- Adsorption, concentration by, 99
- Aëration apparatus, 456, 504, 512
- Albrecht-Hüfner prism, 42
- Albumin (*see also* Protein), 539
- Alcohol, 540
  - determination of water in, 334
- Aldehyde, 540
- Alizarin method, for aluminum, 119
  - for calcium, 139
- Alkali blue, 542
- Alkaloid, 542
- Allantoin, 542
- Alloys, determination of antimony in, 123
  - determination of nickel in, 297
- "Aluminon," 109, 110, 112, 113, 114, 115, 118, 119
  - method for aluminum, 109, 114
- Aluminum, 542
  - alizarin-S method for, 119
  - "aluminon" method for, 109, 114
- Aluminum, determination of, in bearing
  - metals, 114
  - in brass, 114
  - in bronze, 114
  - in minerals, 109
  - in non-ferrous materials, 114
  - in salts, 109
  - in spelter, 114
  - in water, 109, 121
  - hematoxylin method for, 121
- Aluminum chloride, 111
- Amide, 544
- Amine, 544
- Amino-acid (*see also* Nitrogen), 544
- Amino-acid nitrogen, determination of, 467, 515
- Ammonia, 545
  - determination of, 513
  - Nessler's method for, 306
  - phenol-hypochlorite method for, 307
  - preparation of pure, 239
  - strength of, in aqueous solutions, 720
- Ammonia method, for copper, 176
  - for gold, 192
- Ammonium, determination of, in salts, 306, 307
- Amyl alcohol, 550
- Analyses, chemical, hydrogen-ion control
  - in, 216
- Analysis, blood, 443, 469
  - colorimetric, definition of, 1
  - monochromatic, 2, 11
  - nephelometric, definition of, 2
  - photometric, definition of, 1
  - urine, 500, 518
- Aniline, 550
  - chloride method for chlorate, 162
  - determination of, 410
  - dyes, 97
- Aniline method, for furfural, 421

Aniline method, for lead, 257.  
 Anthracene, 550  
 Antimony, 550  
   determination of, in alloys, 123  
   in copper, 123  
   sulfide method for, 123  
 Antimony sulfide, colloidal, 95  
 Antipyrine method for nitrite, 311  
 Apparatus, aëration, 456, 504, 512  
   colorimetric, 10  
   distillation, 452, 501, 505  
 Application, colorimetry, limits of, 8  
 Aqueous solutions, strength of ammonia in, 720  
 Aromatic hydroxy-acids, 551  
 Arsenic, 551  
   mercuric bromide method for, 125  
   quinine arsenomolybdic method for, 128  
   silver nitrate method for, 128  
 Arsenic sulfide, colloidal, 97  
 Atomic weights, table of, 722  
 Aurin tricarboxylic acid, 92, 109, 110, 114, 115  
 Autenrieth-Hellige-Leitz colorimeter, 490

B

Bacteriological work, hydrogen-ion control in, 216  
 Balancing method, calculation of results in, 54  
   color matching, 4, 10  
 Bausch & Lomb-Duboscq colorimeter, 11, 32, 38  
 Bearing metals, determination of aluminum in, 114  
 Beer's Law, 2, 70, 88, 108  
 Bell-Doisy method for magnesium, 264  
 Bell-Doisy-Briggs method for phosphorus, 436  
 Benedict-Theis method for phosphorus, 353  
 Benzaldehyde, determination of, 412  
 Benzoic acid, 552  
 Benzopurpurin, 93  
 Bibliography, colorimetry, 535  
 Bile acids, 552  
 Bile pigment, 552  
   determination of, 479, 480  
 Bile salts, 553  
   determination of, 480  
 Bilirubin, 553  
 Biological fluids, 553

Biological material, determination of iron in, 491  
   determination of nickel in, 298  
 Bismuth, 553  
   iodide method for, 131  
   iodoquinic reagent for, 133  
 Bismuthate method for manganese, 279  
 Bisulfite method for lead, 257  
 Blindness, color, 11  
 Block comparator, LaMotte, 210  
 Blood analysis, 443, 469  
 Blood, 555  
   determination of chlorides in, 159  
   determination of hydrogen ion in, 492, 493  
   determination of magnesium in, 264  
   determination of phosphorus in, 346, 348, 353  
   method of drawing, 443  
 Blood fat, 556  
 Blood filtrate, protein-free, 444  
 Blood serum, determination of calcium in, 142  
 "Blue sky," composition of, 12  
 Bock-Benedict colorimeter, 11, 26  
 Boric acid, 556  
   determination of, 133, 135  
 Boron (*see also* Boric acid) curcumin method for, 135  
   turmeric method for, 133  
 Bottles for color standards, 12  
 Box for Nessler tubes, 13  
 Brass, determination of aluminum in, 114  
 Bread, hydrogen-ion control in, 217  
 Bromate method for gold, 191  
 Bromide method for copper, 181  
 Bromide-peroxide method for gold, 191  
 Bromine, 557  
   determination of, 137  
 Bronze, determination of aluminum in, 114  
 Brown's method for hydrogen ion, 213  
 Brucine, 557  
 Brucine method for nitrate, 318  
 Buffer, 557  
 Buffer action, 201  
 Butyric acid, 558

C

Calcium, 558  
   alizarin method for, 139  
   determination of, in blood serum, 142

- Calcium, determination of, in feces, 146
  - in salt mixtures, 142
  - in urine, 143
- Calcium oleate, method for, 143
- Calculations, 559
  - of results in balancing method, 54
  - in dilution method, 52
  - in duplication method, 53
  - in standard series method, 52
  - simplified method of, 55
  - tables of, 56, 61, 63, 66
- Calibration curves, 70
- Camera, color, 10, 15
- Candy, hydrogen-ion control in, 216
- Canned goods, determination of lead in, 256
- Canning food products, hydrogen-ion control in, 216
- Caramel, 559
- Carbohydrate (*see also*, Dextrose, Glucose, Lactose, and Sugar), 559
- Carbon, 559
  - determination of, in steel, 148
- Carbon dioxide, 562
  - solubility of, in water, 721
- Carbon monoxide, 563
- Care of a precision instrument, 105
- Carnosine, 563
- Carotin, 563
- Cassius, purple of, 99, 191, 194, 196
- Cazeneuve's reagent, 255
- Cerium, 563
- Chemical analysis, hydrogen-ion control in, 216
  - photometric, definition of, 1
  - physico-chemical principles involved, 1
- Chemicals, general, hydrogen-ion control in, 217
- Chile saltpeter, determination of perchlorate in, 163
- China clays, determination of iron in, 237
- Chlorate, 563
  - aniline chloride method for, 162
- Chloride, chromate method for, 159
- Chloride method, for cobalt, 172,
  - for copper, 179
  - for iron, 238
  - for nickel, 303
- Chlorides in blood, determination of, 159
- Chlorine, 563
  - determination of, in water, 157
  - o*-tolidine method for, 157
- Chlorine, permanent standards, 158
  - solubility of, in water, 721
- Chloroform, 565
- Cholesterol, 565
  - determination of, 474, 476
- Chromate, 567
- Chromate method, for chloride, 159
  - for chromium, 167, 169
  - for lead, 254
- Chromium, 568
  - chromate method for, 167, 169
  - determination of, in ores, 167
  - in rocks, 167
  - in steels, 165, 167, 169
  - diphenylsemicarbazide method for, 166
  - König's reagent for, 165
- Chromoisomerization, 89
- Chromoisometric mixtures, 88
- Chromometer, Saybolt, 10
- Citral, 570
  - determination of, in extracts, 414
  - in lemon oils, 414
- Citric acid, 570
- Clays, china, determination of hydrogen ion in, 214
  - determination of iron in, 237
  - determination of titanium in, 384
  - hydrogen-ion control in, 216
- Cleaning processes, hydrogen-ion control in, 217
- Cobalt, 570
  - chloride method for, 172
  - determination of, in paints, 173
  - in varnish, 173
  - extraction method for, 171
  - $\alpha$ -nitroso-  $\beta$ -naphthol method for, 173
- Colchicine, 572
- "Collector," 100, 101
- Colloid, particle size, factors that influence, 102
  - relation of, to color, 97
  - stability, theory of, 95
- Colloidal antimony sulfide, 95
- Colloidal arsenic sulfide, 97
- Colloidal copper sulfide, 97
- Colloidal dyes, 98
- Colloidal ferric oxide, 95, 96, 97
- Colloidal gold, 92, 93, 95, 96, 97, 98
- Colloidal lead sulfide, 97
- Colloidal platinum, 96
- Colloidal tungsten oxide, 101

- Colloidal zinc sulfide, 99
- Colloids, 572
  - color of, 97
  - complex theory of, 96
  - hydrophilic, 92
  - protective, 91
- Color blindness, 11
- Color camera, 10, 15
- Color comparator, 210
- Color, imperfect susceptibility to, 83
  - of colloids, 97
  - relation of colloid particle size to, 97
  - relation of dispersion to, 97
- Color matching, 3
  - balancing method, 4, 10
  - dilution method, 4, 10
  - duplication method, 4, 10
  - standard series method, 3, 10
- Color reactions with titanium, 383
- Color standards, 208
  - bottles for, 12
- Colorimeters, 572
  - Autenrieth-Hellige-Leitz, 490
  - Bausch & Lomb-Duboscq, 11, 32, 38
  - Bock-Benedict, 11, 26
  - directions for using, 103
  - Duboscq, 10, 11, 32, 38, 40, 42, 43
  - for iron, 224, 225
  - for molybdenum, 286, 287
  - for oxygen, 324, 328
  - Frankforter, Walker, and Wilhoit, 328
  - Hilger, 11
  - Kennicott-Campbell-Hurley, 10, 21
  - King, 287
  - Kleinmann, 11, 45
  - Kober, 11, 28, 32
  - Leitz-Duboscq, 11, 40
  - mechanical construction of, 104
  - Meyers, 11
  - Nessler tube, 10, 24
  - Nutting, 2, 11
  - optical construction of, 103
  - Ramsay and Homfray, 324
  - Schmidt & Haensch, 11, 43
  - Schreiner, 10, 18
  - specifications of, 103
  - Spencer-Duboscq, 11, 42
  - Stammer, 10
  - Steiger, 15
  - Stokes and Cain, 225
  - types of, 10
- Colorimeters, wedge type, 48
  - White, 11, 48
- Colorimeter curves, 70, 579
- Colorimeter lamps, 50, 51, 252, 579
- Colorimetric analysis, definition of, 1
- Colorimetric apparatus, 10
- Colorimetric methods, speed of, 7
- Colorimetric tables, 57
- Colorimetry, 579
  - accuracy of, 7
  - Bibliography, 535
  - definition of, 1
  - errors in, 83, 84, 89
  - fallacies in, 84
  - general principles, 1
  - limits of application, 8
  - requirements of, 5
- Columbium, 580
- Comparator block, 210
- Complex theory of colloids, 96
- Concentration, by adsorption, 99
  - by co-precipitation, 100
- Concentration methods, 228
- Congo red, 94
- Conversion table of units, 721
- Copper, 581
  - ammonia method for, 176
  - bromide method for, 181
  - chloride method for, 179
  - determination of, in salts, 181
  - in slags, 176
  - determination of antimony in, 123
  - ferrocyanide method for, 182
  - salicylic acid method for, 183
  - sulfide method for, 178
  - xanthate method for, 184
- Copper sulfide, colloidal, 97
- Co-precipitation, concentration by, 100
- Correction curves, 70
- Crackers, hydrogen-ion control in, 216
- Creatine, 584
  - determination of, 461, 519, 520, 521
- Creatinine, 588
  - determination of 457, 518
- Creatinine zinc chloride, 459
- Cresol, 594
- Cullen lamp, 51
- Cullen's method for hydrogen ion, 492
- Culture media, determination of hydrogen ion in, 214
- Curcumin method for boron, 135

Curves, calibration, 70

Cyanates, 594

Cyanide, 594

determination of, in plants, 151, 154

Prussian blue method for, 151

thiocyanate method for, 154

Cylinders, Hehner, 10

Nessler, 10

Cystine, 595

Cystine, 595

## D

Daylight lamp, 51

Dextrine, 596

Dextrose (*see also* Carbohydrate and Sugar), 596

Diatase, 597

Dihydroxyacetone, 597

Dilution method, calculation of results in, 52

color matching, 4, 10

Dimethylaniline method, for nitrite, 311

Dimethylglyoxime method, for iron, 248

for nickel, 295

Dinitrosalicylic acid, 597

Diphenylbenzidine method for nitrate, 316

Diphenyl carbazide, 255, 258

Diphenyl carbazide method for lead, 255, 258

Diphenylsemicarbazide method for chromium, 166

Dispersion, relation of, to color, 97

Dissociation, electrolytic, 198

Distillation apparatus, 452, 501, 505

Disturbances of equilibria, 87

Di-thio-oxalate method for nickel, 298

Doring's method for gold, 193

Dough, hydrogen-ion control in, 217

Dowsett's method for gold, 194

Drop method for hydrogen-ion determination, 213

Duboscq colorimeter, 10, 11, 32, 38, 40, 42, 43

Duodenal contents, determination of hydrogen ion in, 214

Duplication method, calculation of results in, 53

color matching, 4, 10

Dyes, 597

aniline, 97

aurin tricarboxylic acid, 92

Dyes, benzopurpurin, 93, 98

colloidal, 98

Congo red, 94

determination of, 415

hydrogen-ion control in, 217

methyl orange, 87, 88, 89

methyl violet, 93

Night blue, 93

vat, 97, 415

reduction of, 3, 415

## E

Egg yolk, 598

Eggertz tubes, 10, 13

Electrolytic dissociation, 198

Electroplating, hydrogen ion in, 217

Emulsoids, 94

effect of, 93

Enols, 598

Enzymes, 511, 598

Epinephrine (*see* Adrenaline)

Equilibria, disturbances of, 87

Errors, 83, 598

sources of, 84, 89

Ethyl nitrite, 599

Extraction method for cobalt, 171

Extracts, determination of citral in, 414

determination of hydrogen ion in, 214

determination of vanillin in, 437, 439

Eye strain, 84

Eyes, care of, 106

## F

Farming, hydrogen-ion control in, 217

Fat, 599

Fatigue, 84

Fatty acids, determination of, 474

Feces, determination of calcium in, 146

determination of hydrogen ion in, 214

determination of lead in, 258

Fehling's solution, 433, 434

Fermentation, hydrogen-ion control in, 217

Ferric hydroxide, solubility of, 233

Ferric iron, determination of, 218, 237, 241,

242, 243, 245

Ferric oxide, colloidal, 95, 96, 97

Ferricyanide method for iron, 242

Ferrocyanide method, for copper, 182

for iron, 241

for zinc, 397



- Ferrous iron, determination of, 239, 242, 248  
 Fiske-Subbarow method, for phosphorus, 348  
 Flour, hydrogen-ion control in, 217  
 Fluorine, 599  
     determination of, in rocks, 190  
     titanium sulfate method for, 186  
 Folin and Wu pipette, 446  
 Folin and Wu sugar tube, 470  
 Food, determination of salicylic acid in, 432  
     determination of zinc in, 397  
 Food, products, canning, hydrogen-ion control in, 216  
 Formaldehyde, determination of, 418  
 Frankforter *et al.* colorimeter, 328  
 Fruit juices, determination of hydrogen ion in, 214  
 Furfural, 599  
     aniline method for, 421  
     determination of, 419, 421  
     orcinol method for, 419
- G
- Gardening, hydrogen-ion control in, 217  
 Gases, solubility of, in water, 721  
 Gastric contents, determination of hydrogen ion in, 214  
 Gelatin, hydrogen-ion control in, 217  
 Geranial (*see* Citral)  
 Gland, 600  
 Glassware, composition of, 719  
     lead in, 262  
     resistance tests of, 720  
 Glucose, 600  
 Glue, hydrogen-ion control in, 217  
 Glutamic acid, 601  
 Glutathione, 601  
 Glycerin, 601  
 Glycogen, 601  
 Gold, 601  
     ammonia method for, 192  
     bromate method for, 191  
     bromide-peroxide method for, 191  
     colloidal, 92-8  
     determination of, in ores, 191-96  
         in salts, 196  
         in sea-water, 196  
     Doring's method for, 193  
     Dowsett's method for, 194  
     metaphenylenediamine method for, 192  
     Gold, Prister's method for, 195  
     Rose's method for, 196  
     Gold number, 92
- H
- Hehner cylinders, 10, 14  
 Helianthin, 603  
 Hematin, 94  
 Hematin method for lead, 257  
 Hematoxylin method for aluminum, 121  
 Hemoglobin, 94, 603  
     determination of, 482, 487  
     Hemoglobin values, 486, 487  
 Hemoglobinometer, 482, 483  
 Heroine, 604  
 Hexamethylenamine, 604  
 Hilger colorimeter, 11  
 Histamine, 604  
 Histidine, 604  
 Homogentisic acid, 605  
 Humus, 605  
 Hydrazine sulfate method for phosphorus, 341  
 Hydrochloric acid, 605  
 Hydrogen cyanide, determination of, 151  
 Hydrogen ion, 605  
     concentration of, in water, 204  
     determination of, 205, 208, 210, 213  
         in clays, 214  
         in culture media, 214  
         in duodenal contents, 214  
         in extracts, 214  
         in feces, 214  
         in fruit juices, 214  
         in gastric contents, 214  
         in milk, 214  
         in muscle juice, 214  
         in serums, 214  
         in tissue, 214  
         in urine, 214  
     general theory, 198  
 Hydrogen-ion control, applications of, 216  
     in acid zinc plating, 216  
     in bacteriological work, 216  
     in candy, 216  
     in canning food products, 216  
     in chemical analyses, 216  
     in clays, 216  
     in cleaning processes, 217  
     in crackers, 216  
     in dyes, 217

- Hydrogen-ion control, in electroplating, 217  
 in fermentation, 217  
 in flour, dough, bread, 217  
 in gardening and farming, 217  
 in gelatin and glue, 217  
 in general chemicals, 217  
 in industrial wastes, 217  
 in laundry processes, 217  
 in milk and milk products, 217  
 in paper manufacture, 217  
 in pathological work, 217  
 in pharmaceuticals, 217  
 in pigments, 217  
 in sewage disposals, 217  
 in sugar manufacture, 217  
 in tanning leather, 217  
 in water purification, 217
- Hydrogen-ion determination, Brown's method, 213  
 Cullen's method, 492  
 drop method, 213  
 in blood, 492, 493  
 in urine, 533  
 McClellon's method, 493
- Hydrogen-ion exponent scale, 199
- Hydrogen-ion indicators, 206
- Hydrogen-ion testing set, 213
- Hydrogen peroxide, 608  
 determination of, 332, 333
- Hydrogen sulfide, 609  
 determination of, 375  
 in water, 376  
 methylene blue method for, 375  
 preparation of pure, 240  
 solubility of, in water, 721
- Hydrogen-sulfide method for phosphorus, 344
- Hydrolysis, 610  
 effects of, 87
- Hydrophilic colloids, 92
- Hydroxylamine, 610
- I
- Icteric index, 479
- Illumination "sphere," 51
- Imidazole, 610
- Incinerations, determination of magnesium in, 264
- Indican, 610
- Indicators, 205, 206
- Indigo, 611
- Indole, 611
- Industrial wastes, hydrogen ion in, 217
- Interference eliminators, 96
- Inulin, 611
- Iodide method for bismuth, 131
- Iodine, 611
- Iodoquinic reagent for bismuth, 133
- Ionization, effects of, 87
- Iron, 613  
 acetylacetone method for, 245  
 chloride method for, 238  
 colorimeter for, 224, 225  
 determination of, 491  
 in biological material, 491  
 in china clays, 237  
 in salts, 244  
 determination of manganese in, 273  
 determination of phosphorus in, 342, 344  
 determination of sulfur in, 372, 373  
 determination of titanium in, 377  
 determination of vanadium in, 388, 391  
 dimethylglyoxime method for, 248  
 ferricyanide method for, 242  
 ferrocyanide method for, 241  
 Mellor's method for, 237  
 salicylic method for, 243  
 sulfide method for, 239  
 sulfocyanate method for, 218, 237
- Iron sulfide, solubility of, 233
- Isobutyl alcohol, 620
- Isodisulfocyanic acid, 219, 234
- J
- Julian tubes, 10, 14
- K
- Kennicott-Campbell-Hurley colorimeter, 10, 21
- King colorimeter, 287
- Kleinmann colorimeter, 11, 45
- Kober colorimeter, 11, 28, 32
- König's reagent for chromium, 165
- L
- Lactic acid, 621
- Lactose, 621
- LaMotte block comparator, 210
- Lamps, colorimeter, 50, 51, 252  
 Cullen, 51

- Lamps, daylight, 621  
 Osram-opal, 45  
 Laundry processes, hydrogen ion in, 217  
 Lead, 622  
   aniline method for, 257  
   bisulfite method for, 257  
   chromate method for, 254  
   determination of, in canned goods, 256  
     in feces, 258  
     in solders, 256  
     in tinning baths, 256  
     in urine, 258  
     in water, 257  
   diphenyl carbazide method for, 255, 258  
   hematin method for, 257  
   sulfide method for, 250  
 Lead content of vessels, 262  
 Lead sulfide, colloidal, 97  
 Lead sulfide method for sulfur, 372  
 Lecithin, 626  
   determination of, 478  
 Leitz-Duboscq colorimeter, 11, 40  
 Lemon oils, determination of citral in, 414  
 Levulose, 626  
 Licopin, 626  
 Light, source of, 106  
 Lipoid phosphorus, determination of, 478  
 Lipoids (*see* Lecithin)  
 Lovibond tintometer, 2, 10, 11
- M
- Magnesium, 627  
   Bell-Doisy method for, 264  
   determination of, in blood, 264  
     in incinerations, 264  
     in tissue extracts, 264  
     in urine, 264  
     in water, 266  
   oleate method for, 270  
   phosphomolybdate method for, 266  
 Manganese, 628  
   bismuthate method for, 279  
   determination of, 531  
     in iron, 273  
     in ores, 273  
     in soils, 273  
     in steels, 273  
     in urine, 531  
     in water, 273  
   periodate method for, 273  
   persulfate method for, 271
- Marine organisms, determination of zinc in, 397  
 Mass law, 198  
 McCabe's method for vanadium, 388, 391  
 McClendon's method for hydrogen ion, 493  
 Mechanical construction of colorimeter, 104  
 Mellor's method for iron, 237  
 Mercuric bromide method for arsenic, 125  
 Mercuric sulfocyanate, 220  
 Mercury, 632  
   determination of, in urine, 282  
   sulfide method for, 282  
 Mercury light, 252  
 Metals, bearing, determination of aluminum in, 114  
 Metaphenylenediamine method, for gold, 192  
   for nitrite, 310  
 Methemoglobin, 634  
 Methyl orange, 87, 88, 89  
   standard for titanium, 386  
 Methyl violet, 93  
 Methylene blue method, for hydrogen sulfide, 375  
   for sulfur, 373  
 Milk and milk products, hydrogen ion in, 217  
 Milk, determination of hydrogen ion in, 214  
 Millon's reagent, 431, 432  
 Minerals, determination of aluminum in, 109  
   determination of potassium in, 358  
   determination of tungsten in, 387  
 Molybdate method for phosphorus, 336, 337  
 Molybdenum, 634  
   colorimeter for, 287  
   determination of, in ores, 283  
     in rocks, 283  
     in steel, 292  
     in tungsten, 285  
     in tungsten trioxide, 283  
   peroxide method for, 283  
   tannic acid method for, 284  
 Molybdic acid-quinine method for phosphorus, 343  
 Monochromatic analysis, 2, 11  
 Morphine, 635  
 Morphine sulfate method for formaldehyde, 418

Muscle juice, determination of hydrogen ion in, 214

Myers colorimeter, 11

## N

$\alpha$ -Naphthylamine hydrochloride method for nitrite, 309

Nephelometric analysis, definition of, 2

Nephelometry, definition of, 2

Nessler tubes, 10, 12

Nessler tube box, 13

Nessler tube colorimeter, 10, 24

Nessler tube rack, 13

Nessler's method for ammonia, 306

Nessler's reagent, 96, 306, 636

preparation of, 306, 447, 448

Nesslerization, 96, 317, 449, 451, 506, 508, 510

Nickel, 637

chloride method for, 303

determination of, in absence of cobalt, 300

in alloys, 297

in biological material, 298

in presence of cobalt, 301

in steel, 297, 298

dimethylglyoxime method for, 295

di-thio-oxalate method for, 298

thiocarbonate method for, 296

Night blue, 93

Niobium (*see* Columbium)

Nitrate and nitric acid, 638

Nitrate, brucine method for, 318

determination of, 313, 316-20

in acids, 318

in salts, 318

in sewage, 318, 319

in water, 313, 317-19

diphenylbenzidine method for, 316

phenoldisulfonic acid method for, 313

pyrogallol method for, 319

reduction method for, 317

strychnine sulfate method for, 320

Nitrite and nitrous acid, 649

Nitrite, antipyrin method for, 311

determination of, 308-12

dimethylaniline method for, 311

metaphenylenediamine method for, 310

$\alpha$ -naphthylamine hydrochloride method for, 309

Nitrite, sulfanilic acid and  $\alpha$ -naphthylamine method for, 308

zinc iodide-starch method for, 312

Nitrogen (*see also* Ammonia and Amino-acids), 659

amino-acid, determination of, 467, 515

non-protein, determination of, 446

total, determination of, 500, 502, 505, 506, 508

in urine, 500, 502, 505, 506, 508

Nitro-glycerine, 664

Nitrosodimethylaniline method for perchlorate, 163

$\alpha$ -Nitroso-  $\beta$ -naphthol, 173

$\alpha$ -Nitroso-  $\beta$ -naphthol method for cobalt, 172

Nitro-toluene, 664

Non-ferrous materials, determination of aluminum in, 114

Non-protein nitrogen, determination of, 446

Novocaine, 664

Nutting colorimeter, 2, 11

## O

Observation, photometric, 106

Observer, precautions to be taken by, 106

Oils, 664

lemon, determination of citral in, 414

Oleate method, for calcium, 143

for magnesium, 270

Optical construction of colorimeter, 103

Orcinol method for furfural, 419

Ores, determination of chromium in, 167

determination of gold in, 191-96

determination of manganese in, 273

determination of molybdenum in, 283

Organic substances, 664

determination of, 423

Organisms, marine, determination of zinc in, 397

Ortho-tolidine, 157

Ortho-tolidine method for chlorine, 157

Osmium, 664

Osram-opal lamp, 45

Oxalic acid, 665

Oxybutyric acid, 665

Oxygen, 665

colorimeter for, 324, 328

determination of, in water, 323, 326

solubility of, in water, 331, 721

solubility table for, 331

## P

- Paints, determination of cobalt in, 173  
 Paper manufacture, hydrogen ion in, 217  
 Pathological work, hydrogen ion in, 217  
 Pentosans, 666  
 Pentose, determination of, 426  
 Pepsin, 666  
 Peptization, 97  
 Perchlorate, 666  
   determination of, in Chile saltpeter, 163  
   nitrosodimethylaniline method for, 163  
 Periodate method for manganese, 273  
 "Permutit," 451, 510, 513, 515, 516  
 Peroxide method, for molybdenum, 283  
   for titanium, 377, 384  
 Persulfate method for manganese, 271  
 pH scale, 199  
 Pharmaceuticals, hydrogen ion in, 217  
 Phenol, 666  
   determination of, 428, 429, 431, 481  
   in polluted water, 429  
 Phenoldisulfonic acid method for nitrate, 313  
 Phenolphthalein method for ammonia, 307  
 Phenolsulfonephthalein, 668  
 Phosphate and Phosphoric acid, 668  
 Phosphate, determination of, in presence of silica, 369  
 Phosphomolybdate method for magnesium, 266  
 Phosphorus (*see also* Phosphate), Bell-Doisy-Briggs method for, 346  
   Benedict-Theis method for, 353  
   determination of, 336, 337, 341, 342, 343, 344, 346, 348  
   in blood, 346, 348, 353  
   in cast iron, 342  
   in pig iron, 344  
   in plant extracts, 336, 370  
   in soil, 336, 370  
   in steel, 342, 344  
   in urine, 346, 348  
   in water, 336, 370  
   Fiske-Subbarow method for, 348  
   hydrazine sulfate method for, 341  
   hydrogen sulfide method for, 344  
   molybdate method for, 336, 337  
   molybdic acid-quinine method for, 343  
   vanadomolybdate method for, 342  
 Photometric analysis, definition of, 1  
 Photometric balance, 107  
 Photometric observation, 106  
 Photometry, 11  
 Physico-chemical principles involved in chemical analysis, 1  
 Picric acid, 457, 676  
 Pigments, bile, 676  
   determination of, 479, 480  
   blood, 676  
   hydrogen ion in, 217  
 Pipette, Folin and Wu, 446  
 Pitch, 676  
 Plant extracts, determination of phosphorus in, 336, 370  
   determination of silicon in, 370  
 Plants, determination of cyanide in, 151, 154  
 Platinum, 676  
   colloidal, 96  
   potassium iodide method for, 355  
 Potassium, 677  
   determination of, 357, 359  
   in minerals, 358  
   in soil, 358, 364  
   in water, 358  
 Potassium iodide method, for platinum, 355  
   for selenium, 365  
 Precautions to be taken by observer, 106  
 Precision instrument, care of, 105  
   mechanical construction of, 104  
   optical construction of, 103  
 Principles, general, colorimetry, 1  
 Prism, Albrecht-Hüfner, 42  
 Prister's method for gold, 195  
 Protective action, envelope theory of, 91  
 Protective colloids, 91  
 "Protein error," 95  
 Protein-free blood filtrate, 444  
 Proteins (*see also* Albumin), 677  
   determination of, 527  
 Prussian blue, 678  
 Prussian blue method for cyanide, 151  
 Purple of Cassius, 99, 191, 194, 196  
 Pyrogallol method for nitrate, 319

## Q

- Quinine, 678  
   æsenomolybdic method for arsenic, 128

## R

- Rack for Nessler tubes, 13  
 Ramsay and Homfray colorimeter, 324

- Reagent, Cazeneuve's, 255  
 Reduction method for nitrate, 317  
 Reduction of vat dyes, 3, 415  
 Reflector, double, 104  
   single, 104  
   split, 104  
 Requirements of colorimetry, 5  
 Resorcinol method for zinc, 396  
 Rhodium, 678  
 Rocks, determination of chromium in, 167  
   determination of fluorine in, 190  
   determination of molybdenum in, 283  
 Rose's method for gold, 196
- S
- Saccharine, 678  
 Saffron, 678  
 Salicylic acid, 678  
   determination of, 432, 433  
   in food, 432  
 Salicylic acid method, for copper, 183  
   for iron, 243  
 Salt mixtures, determination of calcium in, 142  
 Salts, bile, determination of, 480  
   determination of aluminum in, 109  
   determination of copper in, 181  
   determination of gold in, 196  
   determination of iron in, 244  
   determination of nitrates in, 318  
 Salvarsan, 681  
 Saybolt chromometer, 10  
 Schmidt & Haensch colorimeter, 11, 43  
 Schreiner colorimeter, 10, 18  
 Sea-water, determination of gold in, 196  
 Selenium, 681  
   potassium iodide method for, 365  
 Sensitiveness, 681  
   variable, 83  
 Serum, blood, determination of calcium in, 142  
   determination of hydrogen ion in, 214  
 Sewage, determination of nitrates in, 318, 319  
 Sewage disposals, hydrogen ion in, 217  
 Silica (*see also* Silicon), 366, 682  
 Silica and phosphate, determination of, in presence of each other, 369  
 Silicate rocks, determination of titanium in, 384
- Silicon (*see also* Silica), 366  
   determination of, in plant extracts, 370  
   in soil, 370  
   in tissues, 366  
   in water, 370  
   sulfite method for, 366  
 Silver, 683  
 Silver nitrate method, for arsenic, 128  
 Skatole, 683  
 Slags, determination of copper in, 176  
 Sodium, 684  
 Sodium alizarin monosulfonate, 119  
 Soil toxicity, 684  
 Soils, determination of manganese in, 273  
   determination of phosphorus in, 336, 370  
   determination of potassium in, 358, 364  
   determination of silicon in, 370  
 Solders, determination of lead in, 256  
 Solubility, of ferric hydroxide, 233  
   of gases in water, 721  
   of hydrogen sulfide in water, 72  
   of iron sulfide, 233  
   of oxygen in water, 331, 721  
   of sulfur dioxide in water, 721  
 Solutions, unbuffered, behavior of, 204  
 Specifications for colorimeters, 103  
 Spectrophotometry, 11  
 Spectroscope, 11  
 Speed of colorimetric methods, 7  
 Spelter, determination of aluminum in, 114  
 Spencer-Duboscq colorimeter, 11, 42  
 Sphere illuminator, 51  
 Stabilizers, colorimetric, 91  
 Stain method for sulfur, 375  
 Stammer colorimeter, 10  
 Standard series method, calculation of results in, 52  
   color matching, 3, 10  
 Standards, 684  
   color, 208  
   bottles for, 12  
   colored glass, 3  
   permanent, 3, 4  
   for chlorine, 158  
   for titanium, 386  
 Starch, 685  
 Steels, determination of carbon in, 148  
   determination of chromium in, 165, 167, 169  
   determination of manganese in, 273  
   determination of molybdenum in, 292

- Steels, determination of nickel in, 297, 298  
     determination of phosphorus in, 342, 344  
     determination of sulfur in, 372  
     determination of titanium in, 377, 384  
     determination of tungsten in, 387  
     determination of vanadium in, 388, 391  
 Steiger colorimeter, 15  
 Stercobilin, 685  
 Stokes and Cain colorimeter, 225  
 Strychnine, 686  
 Strychnine method for vanadium, 393  
 Strychnine sulfate method for nitrate, 320  
 Sugar (*see also* Carbohydrate, Dextrose, Glucose, and Lactose), 686  
     determination of, 469, 471, 472, 524, 525  
 Sugar manufacture, hydrogen ion in, 217  
 Sugar tube, Folin and Wu, 470  
 Sulfanilic acid and  $\alpha$ -naphthylamine method for nitrite, 308  
 Sulfate, 693  
 Sulfide method, for antimony, 123  
     for copper, 178  
     for iron, 239  
     for lead, 250  
     for mercury, 282  
 Sulfite method for silicon, 366  
 Sulfocyanate method for iron, 218, 237  
 Sulfocyanic acid, 221  
 Sulfur, 693  
     determination of, 372, 373  
     in iron, 372, 373  
     in steel, 372  
     lead sulfide method for, 372  
     methylene blue method for, 373  
     stain method for, 375  
 Sulfur dioxide, solubility of, in water, 721  
 Suprarenine (*see* Adrenaline)  
 Syringe, Luer, 496
- T
- Tables, 719  
     colorimetric, 51, 57  
 Tannic acid method for molybdenum, 284  
 Tannin, etc., 698  
     determination of, 435  
 Tanning leather, hydrogen ion in, 217  
 Tantalum, 699  
 Tautomer, 88, 89  
 Thiocarbonate method for nickel, 296  
 Thiocyanate, 699  
 Thiocyanate method, for cyanide, 154  
 Thiocyanate method, for iron, 218, 237  
 Thiophen, 699  
     determination of, 436  
 Thiosulfate, 699  
 Thymol, 699  
 Thymol method for titanium, 381  
 Tin, 700  
 Tinning baths, determination of lead in, 256  
 Tintometer, Lovibond, 2, 10, 11  
     Wesson, 11  
 Tissue, determination of hydrogen ion in, 214  
     determination of silicon in, 366  
 Tissue extracts, determination of magnesium in, 264  
 Titanium, 700  
     color reactions with, 383  
     determination of, 377, 381, 384  
     in clays, 384  
     in iron, 377  
     in silicate rock, 384  
     in steel, 377, 384  
     permanent standard for, 386  
     peroxide method for, 377, 384  
     thymol method for, 381  
 Trypsin, 703  
 Tryptophane, 703  
 Tubes, 10  
     Eggertz, 10, 13  
     Hehner, 10, 14  
     Julian, 10, 14  
     Nessler, 10, 12  
 Tungsten, 704  
     determination of, 387  
     in minerals, 387  
     in steel, 387  
     determination of molybdenum in, 285  
 Tungstic oxide, colloidal, 101  
     determination of molybdenum in, 283  
 Turbidimeter, 704  
 Turbidimetry, 2  
 Turbidity, 704  
 Turmeric method for boron, 133  
 Tyndallmeter, 704  
 Tyramine, 705  
 Tyrosine (*see also* Amino-acid), 705
- U
- Unbuffered solutions, behavior of, 204  
 Units, conversion table of, 721  
 Uranium, 706

Urea, 706  
 determination of, 451, 453, 454, 455, 510, 511  
 Urease, 451, 455, 708  
 Uric acid, 708  
 determination of, 462, 522  
 Urine, 714  
 determination of calcium in, 143  
 determination of hydrogen ion in, 214, 533  
 determination of lead in, 258  
 determination of magnesium in, 264  
 determination of manganese in, 531  
 determination of mercury in, 282  
 determination of phosphorus in, 346, 348  
 determination of total nitrogen in, 500, 502, 505, 506, 508  
 Urine, analysis, 500, 518  
 Urochrome, 714

## V

Vanadio-molybdate method for phosphorus, 342  
 Vanadium, 714  
 determination of, 388, 391, 393  
 in iron, 388, 391  
 in steel, 388, 391  
 McCabe's method for, 388, 391  
 strychnine method for, 393  
 Vanillin, 716  
 determination of, 437, 439  
 in extracts, 437, 439  
 Varnish, determination of cobalt in, 173  
 Vat dyes, 97, 415  
 reduction of, 3, 415  
 Vessels, lead content of, 262

## W

Wastes, industrial, hydrogen ion in, 217  
 Water, 716

Water, determination of, in alcohol, 334  
 determination of aluminum in, 109, 121  
 determination of chlorine in, 157  
 determination of hydrogen ion in, 204  
 determination of hydrogen sulfide in, 376  
 determination of lead in, 257  
 determination of magnesium in, 266  
 determination of manganese in, 273  
 determination of nitrates in, 313, 317, 318, 319  
 determination of oxygen in, 323, 326  
 determination of phenol in, 429  
 determination of phosphorus in, 336, 370  
 determination of potassium in, 358  
 determination of silicon in, 370  
 determination of zinc in, 397  
 purification, hydrogen ion in, 217  
 Weights, atomic, table of, 722  
 Wesson tintometer, 11  
 White colorimeter, 11, 48  
 Wood fiber, 717

## X

Xanthate method for copper, 184  
 Xanthophyll, 717

## Z

Zeolite, 513  
 Zinc, 717  
 determination of, 396, 397  
 in food, 397  
 in marine organisms, 397  
 in water, 397  
 ferrocyanide method for, 397  
 iodide-starch method for nitrite, 312  
 resorcinol method for, 396  
 Zinc plating, acid, hydrogen-ion control in, 216  
 Zinc sulfide, colloidal, 99

















